THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 118 FEBRUARY 1, 1937 No. 2

THE ERYTHROCYTE REACTION OF THE DOG TO COBALT

GEORGE BREWER

From the Department of Physiology, Georgetown University School of Medicine

Received for publication May 26, 1936

All published reports on the effect of cobalt upon the number of red blood cells seem to indicate that this metal produces a rather marked erythrocytosis. The rapidity with which this response occurs depends upon the amount fed. Most of this data has been obtained upon the albino rat (1, 3, 4). In this animal as little as 2.5 mgm./kilo of body weight of cobalt has sufficed to bring about a 50 per cent increase in the number of erythrocytes. In the dog it has been claimed (2) that 1.0 to 1.5 mgm. every two days causes polycythemia. Other animals such as the guinea pig, mouse, frog (8) and the rabbit (9) upon which the effect of this metal has been tested have been given massive doses. Polycythemia occurred also in these species but in addition there was—depending on dosage—anorexia, cessation of growth, loss of weight, loss of fertility, diarrhea, lethargy and death.

The mechanism for the production of the erythrocytosis seems most likely due to stimulation of the bone marrow. However, the possibility that the spleen as an organ for erythrocyte destruction has this function depressed by cobalt has recently (3, 4) been suggested. This latter investigation however, was carried out on the albino rat, a notoriously poor animal for blood studies because of the hematological disturbances due to Bartonella infection—so frequent in the rat.

Inasmuch as but one investigation (2) was found in the literature describing the reaction of the dog to cobalt, it was thought worth repeating. It was planned to study also the possibility of the involvement of the spleen in the expected erythrocytosis.

Four healthy young adult dogs previously freed of intestinal worms were used in the experiment. Two of the dogs, H and A, were splenectomized about a month before the beginning of observations. Recovery from the operation was prompt and uneventful.

To insure uniform metabolic conditions the animals were fed Cowgill's (5) synthetic diet supplemented by Karr's (6) salt mixture so as to receive about 65 calories per kilo of body weight per day. Fat, in the form of lard, 17 grams, and unsalted butter, 9 grams per 100 grams of the mixture, were added at the time of each feeding. Weight for each animal remained constant over the period of observation.

The cobalt was administered as $CoCl_2 \cdot 6H_2O$, powdered and thoroughly mixed with a portion of the control diet. The dosage originally given was the equivalent of 0.8 mgm. cobalt metal per kilo of body weight per day. This is approximately the amount given by Mascherpa (2) who administered 1.5 mgm. of the powdered metal every other day. Subsequently, the dosage was increased to the equivalent of 4.0 mgm. The effective dose in the rat seems to be approximately 2.5 mgm. per kilo. During the oral administration of cobalt none of the animals showed any toxic symptoms.

Throughout the course of the observations erythrocyte counts were made every two or three days. Blood was obtained by venepuncture of the shaved ear. When flowing freely, blood was drawn to the 1.5 mark of the usual Thoma pipette. Dilution 1 to 200 was then made with Hayem's solution, the mixture shaken and the cells counted in a chamber of the Levy-Hausser type with the improved Neubauer ruling.

Daily erythrocyte counts on normal untreated dogs on a constant diet in this laboratory show a rather large variation—as high as 15 per cent. This same order of magnitude of variation in erythrocyte count has also been observed by Wintrobe (10) and by Mayerson (11).

The magnitude of this daily normal fluctuation is of extreme importance, inasmuch, as in the only previous report (2) on the effect of cobalt on the erythrocyte count in the dog, variations of this degree were assumed to indicate a polycythemia, provided they occurred at the right stage during the course of an experiment.

The results of the present investigation are summarized in table 1.

TABLE 1

Effect of cobalt on erythrocyte count

DOG	CONTROL PERIOD 14 DAYS		0.8 MGM. COBALT 16 DAYS		4.0 MGM. COBALT 26 DAYS	
	Variation	Mean	Variation	Mean	Variation	Mean
H	5.17–5.29	5.23	5.20–5.33	5.25	5.30–5.62	5.43
A	4.95–5.60	5.25	4.90–5.68	5.42	5.06–5.46	5.29
B	6.19–6.95	6.52	6.36–6.98	6.63	6.42–6.91	6.65
L	6.55–6.96	6.70	6.55–7.17	6.88	6.52–7.36	7.04

The figures represent number of erythrocytes per cubic millimeter in millions.

Following the failure to produce a polycythemia in either normal or

splenectomized dogs by dietary feeding of cobalt it was thought worth while to attempt to bring about the phenomenon by injecting even larger doses subcutaneously, inasmuch as this method is said to be successful in the rat (3, 4, 1). The rare chance that the cobalt was not absorbed from the intestine in the dog could not be eliminated.

Accordingly, cobalt as a solution of $CoCl_2 \cdot 6H_2O$ was injected in doses of 15 mgm. per kilo of body weight every other day in dog H and dog L. After two injections tender swollen areas at the sites of injection were noted and after the third treatment extensive sloughs of the skin of the back and shoulders had developed which necessitated cessation of this form of cobalt administration. Incidentally, the erythrocyte count did not rise during the course of these injections, nor during the subsequent two weeks; apparently the reactions of the rat and dog to the subcutaneous administration of cobalt chloride are quite different.

DISCUSSION AND SUMMARY

Concerning the polycythemia described in the dog on doses of 1.5 mgm. per kilo every other day it is this author's opinion that no polycythemia actually occurred; variations of 15 per cent to 20 per cent between cobalt fed animals and controls, and just as great variations occurring from time to time in the controls, do not constitute evidence for specific effect.

In the present experiments when the daily dose of cobalt was increased five times over that claimed (2) to cause polycythemia in the dog and twice that having the same result in the rat no appreciable erythrocytosis was even then observed. However, the possibility is still entertained that sufficiently great increase in the daily feeding of cobalt may increase the erythrocyte concentration in the dog because of the fact that this response is said to be so unequivocal in certain species. Whether the amount of cobalt required to bring about this increase would result in toxic symptoms is not yet known. This point is still under investigation in this laboratory.

In the present investigation it was found that cobalt polycythemia does not occur in the dog on doses formerly claimed to have this result. Furthermore, doses about twice those causing 50 per cent increase in number of red cells in the rat do not produce any erythrocytosis in the dog.

Subcutaneous injection of cobalt chloride, while apparently harmless in the rat, results in extensive destruction of tissue and slough in the dog.

The splenectomized animals remained relatively anemic throughout the course of the observation; otherwise their response was the same as that of the unsplenectomized animals.

REFERENCES

(1) Orten, J. M. This Journal **114:** 414, 1936.
(2) Mascherpa, P. Arch. Ital. di Biol. **82:** 112, 1930.

(3) BERWALD, W. P. E., J. H. ARSENEAU AND M. S. DOOLEY. Proc. Soc. Exper. Biol. and Med. **32:** 430, 1934.
(4) ORTEN, J. M. Proc. Soc. Exper. Biol. and Med. **32:** 1091, 1935.
(5) COWGILL, G. R. J. Biol. Chem. **56:** 725, 1923.
(6) KARR, W. G. J. Biol. Chem. **44:** 225, 1920.
(7) ORTEN, J. M., F. A. UNDERHILL, E. R. MUGRAGE AND R. C. LEWIS. J. Biol. Chem. **96:** 11, 1932.
(8) SUTTER, J. Compt. Rend. Soc. Biol. **116:** 994, 1934.
(9) KLEINBERG, W. This Journal **108:** 545, 1934.
(10) WINTROBE, M. M. Personal communication.
(11) MAYERSON, H. S. Anat. Record **47:** 239, 1930.

A COMPARISON OF ORAL ADMINISTRATION VERSUS INTRAPERITONEAL INJECTION OF COLLOIDAL IRON UPON BLOOD REGENERATION IN NUTRITIONAL ANEMIA OF THE RAT

HOWARD H. BEARD AND THOMAS S. BOGGESS

From the Department of Biochemistry, Louisiana State University Medical Center, New Orleans

Received for publication July 20, 1936

Experimental evidence for and against the effectiveness of inorganic Fe alone in nutritional anemia of the rat has been published by a number of workers. Negative results seemed to show that Fe alone cannot be utilized for blood regeneration. Positive results were supposed to be due to Cu contamination of the milk or Fe solution used, or to an Fe-Cu storage in the animal. Fe and Cu storage does not occur in the severely anemic animal and cannot be responsible for the positive results obtained when Fe alone is fed. Many years' study of these and other criticisms of positive Fe results have convinced us that inorganic Fe alone can be very effectively utilized in blood regeneration and that these results are not due to Cu contamination from the cages, milk or Fe solution used (1, 2, 3, 4).

Failure of blood regeneration when Fe alone is administered may be due to several factors, i.e., lack of a quantitative Fe and milk intake; lack of Fe absorption; solubility and form of Fe salt used; pH of the gastro-intestinal tract; storage and excretion of Fe, etc. From the clinical point of view the question of Fe absorption has always been a factor of major importance. Ferrous salts are believed to be much better absorbed and utilized than ferric salts. We have used ferrous chloride in over 90 per cent of our anemic animals and can show a quantitative difference in absorption and utilization of daily doses of Fe varying from 0.05 to 0.25 mgm., as judged by the subsequent reticulocyte response (1). It should be remembered that most workers who have obtained negative results with Fe alone in anemic rats have fed ferric chloride by mouth.

It has been our belief for some time that negative results with Fe alone might possibly be due to its lack of absorption from the intestinal tract. In this paper we will report a study of the total amount of colloidal Fe necessary to bring about blood regeneration (4 to 13.5 grams Hb per 100 cc.) when given by mouth as compared to that injected into anemic rats

together with the per cent utilization of Fe upon Hb formation in these cases.

EXPERIMENTAL. Normal young rats were placed upon cow's milk when they were 23 days of age. They never had access to their mother's diet at any time. The milk used was that supplied to Charity Hospital. It is milked into glass containers, poured into a glass lined cooler, pumped into glass lined tank cars and shipped to New Orleans. The Cu content of this milk as determined by the diethyl dithiocarbamate method at different times averaged 0.11 mgm. per liter. We have also used milk

TABLE 1

Comparison of oral administration versus injection of colloidal Fe upon blood regeneration

GROUP	NUMBER OF RATS	BODY WEIGHT		Hb PER 100 CC.		RED BLOOD CELLS PER C. MM.		DURATION OF EXPERIMENT		DIET
		Before	After	Before	After	Before	After	Hb	Red blood cells	
		grams	*grams*	*grams*	*grams*	*millions*	*millions*	*weeks*	*weeks*	
A	5	49	93	4.1	13.2	2.4	7.6	3	3	1
B	5	77	106	4.0	13.3	2.9	7.6	4	4	2
C	6	95	133	4.9	14.2	3.8	9.5	5.5	5.5	3
D	8	65	98	4.0	14.1	2.4	7.5	3	3	4
E	5	73	122	4.4	15.7	2.8	7.3	4.2	3.4	5
F	5	76	125	4.5	15.2	2.7	7.0	4.8	4.8	6

Diet 1. Milk + 2.0 mgm. colloidal Fe daily by mouth. (Total Fe 42 mgm.)
Diet 2. Milk + 2 mgm. colloidal Fe injected per week. (Total Fe 8 mgm.)
Diet 3. Milk + 0.26 mgm. colloidal Fe daily by mouth. (Total Fe 10 mgm.)
Diet 4. Milk + 0.26 mgm. collodial Fe injected every other day. (Total Fe 2.73 mgm.)
Diet 5. Milk + 2 mgm. colloidal Fe and 0.40 mgm. colloidal Cu injected per week.
Diet 6. Milk + 2 mgm. colloidal Fe and 0.40 mgm. colloidal Mn injected per week.

directly from the cow that had not come in contact with metal both in Cleveland and in New Orleans with identical results.

Hb estimations and erythrocyte counts were made weekly by the technique described in our original paper (1). New galvanized iron cages covered with aluminum paint were used in all studies. The Fe solution used was the "Eisen-Diasporal," ($Fe_3O_4 + 4H_2O \rightarrow 2Fe(OH)_3 + Fe(OH)_2$), a highly dispersed colloidal ferrous and ferric hydroxide preparation in glucose of the Klopfer Chemical Works of Dresden. Thus glucose is the only substance besides iron that is present in this preparation and it is very unlikely that it had any effect on blood regeneration. This preparation is Cu-free. Also no Cu could be detected in a 150 cc. sample of it

by the carbamate method. The 3 cc. of sterile solution in each vial contained 4 mgm. Fe. The Fe-Cu diasporal was used for group E, and the Mn-diasporal was added to the Fe diasporal for group F. Intraperitoneal injections of 2 mgm. Fe were made in some anemic rats weekly or 0.26 mgm. every other day. The control anemic rats received these amounts added daily to the milk. No toxicity occurred in any of the animals reported in this study.

RESULTS AND DISCUSSION. The average results obtained upon blood regeneration are given in table 1. The total dose of Fe fed to group A and injected in group B was larger than necessary for blood regeneration. The study was then repeated by injecting and feeding smaller doses. Group C recovered when 0.26 mgm. colloidal Fe was fed daily by mouth for 5.5 weeks, total Fe, 10.0 mgm. In our original experiments in Cleveland (1) it took a total of 10.5 mgm. of Fe (H_2S filtrate of electrolytic Fe) in 6 weeks for recovery. Therefore, our animals absorb and utilize 0.25

TABLE 2

Showing percentage utilization of colloidal Fe by mouth upon Hb formation

(Blood volume, 7 cc.; 0.335 mgm. Fe = 100 mgm. Hb)

GROUP	NUMBER OF RATS	TOTAL Fe FED (1)	ACTUAL Hb OUTPUT OVER ANEMIC LEVEL (2)	Fe ABSORPTION (AS Hb) FROM ACTUAL Hb OUTPUT OVER ANEMIC LEVEL (2) (3)	RATIO OF Fe ABSORPTION (AS Hb) (3) TO Fe INTAKE (1) (4)
		mgm.	*mgm.*	*mgm.*	*per cent*
A	5	42.0	774.8	2.59	6.2
C	6	10.0	649.1	2.18	21.8

mgm. ferrous and ferric Fe daily for blood formation, either in the colloidal or crystalloidal form, at about the same rate. Group D recovered when 0.26 mgm. colloidal Fe was injected every other day for 3 weeks, total Fe 2.73 mgm.

Even though our Fe solution was Cu-free, nevertheless we wished to study the effect of added Cu, and also Mn, with Fe, as compared to the Fe effect alone. Groups E (Fe-Cu) and F (Fe-Mn) gained slightly more Hb than the control group on Fe alone but since this group showed an average normal Hb content (13.2 g/100 cc.) in the same length of time as the Fe-Cu and Fe-Mn groups, it is seen that these elements do not necessarily assist in the transformation of Fe into Hb in the body. The increase in erythrocytes is certainly not influenced by injected Cu or Mn.

The data given in table 2 show an average increase from 6 to 22 per cent utilization of Fe upon Hb formation when the total dose of Fe was 42 and 10 mgm. respectively. In this connection Eichholtz and Unrath (5) observed a 25 per cent absorption of ferrous salts in mice. This was

4 to 5 times as great as the absorption of ferric chloride and 15 times as great as the absorption of reduced Fe.

The data given in table 3 show the average percentage utilization of injected colloidal Fe upon Hb regeneration. (Blood volume (6) = 7 cc.; 100 mgm. Hb = 0.335 mgm. Fe.) There are 2.37 mgm. Fe contained in 708 mgm. Hb increase in group D. There was an average utilization of 28 per cent for the 8 mgm. dose and 87 per cent for the 2.73 mgm. dose. This dose would be equivalent to 0.13 mgm. daily, and probably represents the minimum daily dose of injected colloidal Fe necessary for Hb regeneration in the anemic rat under the conditions of these studies.

Eveleth, Bing and Myers (7) injected pure Fe into their anemic animals. Complete regeneration of Hb and erythrocytes was obtained in 7 weeks with 0.5 mgm. doses of Fe, injected on alternate days, total Fe 12.3 mgm. The injection of Cu with Fe had no better effect on blood regeneration than the injection of the same dose of Fe alone. They

TABLE 3

Showing percentage utilization of injected colloidal Fe upon Hb formation

(Blood volume, 7 cc.; 0.335 mgm. Fe = 100 mgm. Hb)

GROUP	NUMBER OF RATS	TOTAL Fe INJECTED (1)	ACTUAL Hb OUTPUT OVER ANEMIC LEVEL (2)	THEORETICAL Hb OUTPUT FROM TOTAL Fe INJECTED (1) (3)	RATIO OF ACTUAL Hb OUTPUT (2) TO THEORETICAL Hb OUTPUT (3) (4)
		mgm.	*mgm.*	*mgm.*	*per cent*
B	5	8.0	661.5	2,388.0	27.7
D	8	2.73	708.0	814.9	86.9

stated that Cu may assist in the absorption of Fe from the intestinal tract. In a former study Bing, Saurwein and Myers (8), using milk containing 0.14 mgm. Cu per liter, observed that the injection of 0.5 mgm. Fe per day for 17 days (total Fe, 8.5 mgm.) gave nearly complete blood regeneration in that time, the results being essentially the same as those obtained by giving 0.5 mgm. Fe plus 0.025 mgm. Cu daily by mouth for 17 days.

Starkenstein (9), Kahn (10), Mitchell (11), Lintzal (12), Binet and Strumza (13), Muntwyler and Hanzal (14), and others have reported the beneficial effect of Fe in blood regeneration in experimental anemias. Fog (15) reported Hb recovery in anemic rats depending upon the daily dose of Fe fed from 0.125 to 2 mgm. These results are identical with those reported by Beard and Myers (1).

In spite of the fact that the etiology of hypochromic anemia in man and hemorrhagic anemia in the dog is somewhat different from that of nutritional anemia in the rat, nevertheless all these anemias respond to Fe alone. For instance Meyer (16) reported the beneficial effect of colloidal

Fe in hemorrhagic anemia in dogs. Whipple and Robscheit-Robbins (17) injected colloidal Fe into dogs with hemorrhagic anemia and observed an average utilization of Fe of 105 per cent on Hb production. Heath, Strauss and Castle (18) reported an average utilization of 96 per cent when colloidal Fe was injected into patients suffering from hypochromic anemias.

The results discussed above show that inorganic Fe alone is very effective in blood regeneration in nutritional anemia of the rat. Before negative results with Fe should be accepted it should be injected in optimum doses in a sufficient number of anemic rats. If this is done it is certain that blood regeneration will be rapid.

SUMMARY

Young weanling rats were made anemic by feeding on cow's milk containing an average of 0.11 mgm. Cu per liter. They were then fed or injected with different amounts of a colloidal Cu-free Fe preparation until blood regeneration was complete. The total amount of Fe required under these conditions was then compared and the per cent utilization of Fe in Hb formation determined.

Two milligrams colloidal Fe injected weekly for 4 weeks (8 mgm.) was as effective on blood regeneration as 2 mgm. ingested daily by mouth for 3 weeks (42 mgm.). Twenty-six hundredth milligram injected every other day for 3 weeks (2.73 mgm.) was as effective as the ingestion of 0.26 mgm. daily for 5.5 weeks (10 mgm.).

The utilization of colloidal Fe by mouth on Hb formation was 6 per cent for the 42 mgm. dose and 22 per cent for the 10 mgm. dose. These values for the injected doses were 28 per cent for the 8 mgm. dose and 87 per cent for the 2.73 mgm. dose. It was estimated that 0.13 mgm. is the minimum daily dose of injected Fe required for Hb regeneration under the conditions of these experiments.

The injection of Fe and Cu, or Fe and Mn, gave no better results on blood regeneration than the injection of the same dose of Fe alone.

We wish to thank the Doak Chemical Company of Cleveland and the Klopfer Chemical Works of Dresden for the supply of the colloidal metals used in this study.

REFERENCES

(1) Beard, H. H. and V. C. Myers. J. Biol. Chem. **94:** 71 1931.
(2) Andes, E. J. and H. H. Beard. This Journal **108:** 91, 1934.
(3) Beard, H. H. and E. J. Andes. This Journal **109:** 316, 1934.
(4) Beard, H. H. and T. S. Boggess. This Journal **113:** 642, 1935.
(5) Eichholtz, F. and H. Unrath. Arch. Exper. Path. u. Pharmakol. **178:** 154, 1935.
(6) Donaldson, H. H. The rat. Memoirs of the Wistar Institute of Anatomy and Biology, no. 6, p. 84, Philadelphia, 1915.

(7) EVELETH, M. W., F. C. BING AND V. C. MYERS. J. Biol. Chem. **101:** 359, 1933.
(8) BING, F. C., E. M. SAURWEIN AND V. C. MYERS. J. Biol. Chem. **105:** 343, 1932.
(9) STARKENSTEIN, E. Ergbn. d. ges. Med. **14:** 607, 1929–30.
(10) KAHN, R. G. Med. Klin. **25:** 52, 1929.
(11) MITCHELL, H. H. Illinois Agric. Exper. Sta., Ann Reports, p. 89, 1933.
(12) LINTZAL, W. Ztschr. f. Biol. **87:** 97, 1928.
(13) BINET, L. AND M. V. STRUMZA. Sang **8:** 1041, 1934.
(14) MUNTWYLER, E. AND R. F. HANZAL. Proc. Soc. Exper. Biol. and Med. **30:** 845, 1933.
(15) FOG, E. E. Hospitalstidende **77:** 284, 1934.
(16) MEYER, A. E. J. Lab. and Clin. Med. **18:** 1127, 1933.
(17) WHIPPLE, G. H. AND F. S. ROBSCHEIT-ROBBINS. Am. J. Med. Sci. **191:** 11, 1936.
(18) HEATH, C. W., M. B. STRAUSS AND W. B. CASTLE. J. Clin. Invest. **11:** 1293, 1932.

ACID PRODUCTION IN THE FUNCTIONING HEART UNDER CONDITIONS OF ISCHEMIA AND OF CONGESTION

ROBERT M. MOORE AND MAX M. GREENBERG

From the Departments of Surgery and of Biochemistry, University of Texas, Medical Branch, Galveston

Received for publication August 6, 1936

A former series of experiments demonstrated the sensitivity of pain-endings to a slightly acid reaction and suggested that the accumulation of acid metabolites in tissues deprived of arterial circulation serves as a stimulus to initiate the pain which accompanies ischemia (Moore, Moore and Singleton, 1934). To test this possibility it is necessary to know the degree of acid reaction attained in ischemic tissues. For this purpose we have determined, in a series of animals, the pH of blood obtained from the veins of the functioning heart at varying intervals after ligation of the coronary arteries. As a parallel study and as one form of control, determinations were also made after periods of venous congestion. Furthermore, certain changes in the behavior of the heart under conditions of ischemia and of congestion were noted.

METHOD. The experiments were performed upon cats and dogs anesthetized with dial. Under artificial respiration the ventral halves of the right and left fifth ribs were removed and the sternum transected. The pericardium was opened widely to expose the entire ventral surface of the heart. By tilting the apex of the organ forward a good view of the dorsal surface was obtained.

A ligature was passed beneath the stem of each coronary artery proximal to its first major branch. When both arteries were ligated at this level the ventricles were deprived of virtually their entire supply of arterial blood, the only arterial twig proximal to the ligatures being a small recurrent branch given off by one or both trunks to supply the cone of the pulmonary aorta.

After ligation of both coronary arteries the contractions of the heart rapidly emptied all blood from the coronary veins. Therefore, in order to obtain a sample of the coronary venous blood after the prescribed period of ischemia, it was necessary to confine blood in the veins by ligating the chief tributaries of the coronary venous sinus. Ligation of the left coronary vein ("great cardiac vein") and of the smaller right coronary vein (Gross, 1921) served to trap enough blood in the vascular channels

of the ventricles to permit collection of one adequate sample. It was often impossible to obtain more than one sample, however, and for this reason vena cava samples were used as controls.

After exposure of the heart the procedure consisted of the following steps: 1, placing of the ligatures beneath arteries and veins; 2, ligation of the arteries, followed immediately by 3, ligation of the veins, and 4, after the heart had functioned for a given period, collection of a blood sample from the left coronary vein followed by collection of a control sample from the inferior vena cava. The blood samples were taken at the moment when it appeared that ventricular activity was about to cease. They were collected under oil, allowing the blood to be propelled through the needle into the syringe by the force of the cardiac impulse. It was possible thus to obtain bubble-free samples of coronary venous blood averaging 0.5 cc. in volume in large cats and 1.0 cc. in large dogs. In some animals a second sample of coronary blood was obtained after neutral saline had been injected down the left coronary artery to displace blood from the small vessels into a large vein suitable for aspiration. As a rule this second sample exhibited the greater chemical change.

The effects of venous congestion were studied in a separate series of animals in which all the tributaries of the coronary venous sinus were ligated and also the small veins which empty directly into the right auricle at its anterior border. In these experiments the coronary veins became greatly engorged and venous blood samples of large volume could be obtained.

For determination of pH the blood samples were placed under oil in special centrifuge tubes (3 mm. x 11 mm.) containing purified, washed sodium fluoride as anticoagulant and preservative. The samples were centrifuged within one-half hour after collection, and pH determinations were made upon the plasma in duplicate or triplicate.

The type of micro-quinhydrone-electrode described by Cullen (1929) was used for pH determinations. A drop of a saturated solution of quinhydrone in redistilled acetone was placed upon the platinum wire, where the quinhydrone adhered upon evaporation of the acetone. The apparatus was then assembled and placed in the air bath and brought to the temperature of the calomel and standard cells. The desired amount of plasma, about 0.05 cc., was then drawn from the centrifuge tube into the capillary chamber of the apparatus, taking care that no oil entered the chamber. The filled capillary end of the chamber was immediately inserted below the surface of a saturated KCl solution in a beaker connected with the calomel electrode and acting as a bridge. The lead of the electrode was connected to a potentiometer, and the E. M. F. read at 30 second intervals for 2 to 3 minutes. E. M. F. was converted into pH by means of the formula (Kolthoff, 1931):

$$\text{pH} = \frac{0.4532 - 0.00009\,(t - 25) - E}{0.0591 + 0.0002\,(t - 25)}$$

Often slight drifts of E. M. F. were encountered, not exceeding 2 to 3 mv. and not affecting the value of the results. In a few cases the drift in potential was from 5 to 10 mv. In most such cases experimental and control samples exhibited the same drift, indicating a similar error in both, and the average potentials for the three-minute periods were used in calculating the pH. When only one sample of an experiment showed a large drift the experiment was discarded. Preliminary tests on plasma of dog's blood, in which the micro-electrode was compared with a glass electrode, indicated that the micro-electrode was quite satisfactory for the determination of the pH of plasma.

Lactic acid was determined by the method of Mendel and Goldscheider (1925). This method was checked by adding known amounts of lactic acid to blood and determining the percentage recovery and by comparison with the manometric method of Avery and Hastings (1931). It was found to be satisfactory for blood or plasma containing 30 mgm. per cent or more of lactic acid.

Results and discussion. The effects of ischemia were in striking contrast to those of congestion. Ligation of both coronary arteries invariably resulted in interruption of the heart's function within a period measured in minutes. In every animal an active corneal reflex was present at the moment the arteries were ligated. In the cat the auricles continued to maintain a slow, forcible and regular beat, even for some minutes after the ventricles had ceased contracting. There was no visible change in the ventricular beat for at least 2 minutes. After 2 to 5 minutes ventricular fibrillation intervened in 11 of 18 cats, the auricles still maintaining an apparently normal rhythm. In the dog ventricular fibrillation occurred within 1 or 2 minutes. At about this time both dogs and cats exhibited pseudaffective reflexes, such as movements of the head, movements of the extremities, and deep respiratory movements, indicating a stimulation of pain-endings. Thereafter the corneal reflex was abolished and voluntary respiratory movements ceased. After 2 to 3 minutes more the ventricles underwent rapid dilatation and soon the ventricular beat became imperceptible.

In contrast, when only the coronary veins were ligated, no arrhythmia nor dilatation nor failure resulted. Both auricles and ventricles maintained a slow, regular beat throughout the duration of the experiment which was over 40 minutes in one instance. The corneal reflex remained active and there was no pseudaffective indication of painful stimulation. The heart assumed a reddish, purple hue with the surface moist and arteries and veins prominent, in contrast to the dry, pallid appearance of

the hearts in which the arteries had been ligated. Within 5 minutes after venous ligation the heart took on a silvery sheen from the collection of a colorless liquid beneath the epicardium overlying the chief vessels. Soon this liquid effused upon the surface where it gathered in large drops and puddles until it trickled down the sides of the organ. By the end of a twenty-minute period of congestion the heart literally "wept" and the blood-free transudate accumulated in the pericardial sac.

This contrast between the effects of ischemia and of congestion was borne out by the chemical determinations. Samples of coronary venous blood taken after ligation of both coronary arteries showed a much lower pH than did vena cava samples taken at the same time. The actual period of ischemia, i.e., the interval between ligation of the arteries and taking of the samples, varied from 3 minutes to 9 minutes in the cat and from 2 minutes to 4 minutes in the dog. In the series of 18 cats the pH of the blood from the cardiac vein varied from 7.11 to 6.40, the median value being pH 6.92, whereas the pH of the vena cava blood ranged from 7.64 to 7.15 with a median pH value of 7.39. In every animal the pH of the coronary blood was much lower than that of the vena cava blood, the actual difference varying from 0.22 to 0.81, exceeding 0.66 in six animals and amounting to 0.75 or more in four of these six. Similarly, in 5 successful arterial ligation experiments in dogs the pH of the coronary blood varied from 7.20 to 6.93, the median value being 7.14, whereas the pH of the vena cava samples in the same animals varied from 7.49 to 7.30 with a median value of 7.40. Again, in every animal the pH of the coronary blood was lower, the actual difference ranging from 0.22 to 0.38.

When only the veins were ligated, however, the difference in pH between coronary and vena cava samples was slight. In 5 cats the pH of coronary venous blood taken 10 to 20 minutes after ligation of the coronary veins varied from 7.19 to 7.34, the median value being pH 7.27, while in the same animals the pH of vena cava blood taken at the same time varied from 7.25 to 7.44 with a median value of pH 7.38. In every animal the pH reading for the coronary blood was the lower, the difference varying from 0.06 to 0.15 and the median difference being 0.10. This difference is small and of doubtful significance.

Under normal conditions the blood of the coronary veins is certainly no more acid than is coronary blood obtained under conditions of congestion. Since the difference between the pH of the vena cava blood and that of coronary blood in the congestion experiments was very small it is reasonable to assume that arterial ligation did produce a marked lowering in the pH of the coronary blood, amounting, practically, to the difference observed between the pH of the vena cava sample and that of the sample removed from the coronary vein at a given interval after ligation of the coronary arteries. In this regard the following experiment is of interest. Ligatures were tied about the coronary arteries but the heart

continued to beat in an apparently normal fashion and became congested rather than pallid as in the usual ischemia experiment. After 10 minutes it was discovered that the ligature about the left coronary artery had been placed distal to its large circumflex branch which still carried blood to the heart. A coronary venous sample taken at this juncture was of pH 7.27 whereas a vena cava sample was of pH 7.38. This difference of 0.11 was similar to that observed in the simple congestion experiments. The circumflex artery was then ligated. Within a few minutes the heart became pale and after 4 minutes the ventricles dilated. Seven minutes after ligating the circumflex vessel another coronary sample was taken having a pH of 6.92 whereas a control sample taken from the vena cava a moment later was of pH 7.38. It is obvious that this difference represents a lowering of the pH of the coronary venous blood amounting practically to 0.46.

It has been noted that while in normal states cardiac muscle utilizes lactic acid, it produces the acid under anoxemic conditions (Katz and Long, 1925; McGinty and Miller, 1933; Himwich, Goldfarb and Nahum, 1934; Kuhl, 1934; Grayzel, Tennant, Stringer and Sutherland, 1934). An indication of the degree of anoxemia necessary for the alteration of the metabolism of cardiac muscle to the lactic-acid producing stage is found in comparing the two series of ligations. Thus, in 6 cats in which samples were taken 20 minutes after ligation of the coronary veins, the lactic-acid content of the coronary blood varied from 24 to 66 mgm. per cent, averaging 48 mgm. per cent, whereas the vena cava blood contained 34 to 84 mgm. per cent and averaged 55 mgm. per cent. In contrast, in 8 cats in which samples were taken 5 to 9 minutes after ligation of the coronary arteries the lactic-acid content of the coronary blood varied from 70 to 230 mgm. per cent and averaged 129 mgm. per cent, while the lactic-acid content of the vena cava blood varied from 16 to 73 mgm. per cent and averaged only 42 mgm. per cent. Similarly, in 3 dogs in which the arteries had been ligated, the lactic-acid content of the coronary blood averaged 91 mgm. per cent as compared with an average content of 55 mgm. per cent in the control samples from the vena cava.

No absolutely constant relationship could be drawn between the duration of ischemic contraction and the extent of pH change. However, of six cats in which the coronary samples were taken less than 6 minutes after ligation of the arteries only two showed a lowering of pH exceeding 0.40. In contrast, of twelve cats in which the arteries had been ligated for 6 minutes or more before the taking of samples ten showed a lowering of the pH of the coronary blood exceeding 0.40, and this group included five of the six animals which exhibited a fall in excess of 0.66. Similarly, in the five dogs the samples were taken 2 to 4 minutes after ligation of the arteries, and the maximum pH change for this short period was 0.38.

There was no apparent difference in the rate of acid-production after

the onset of ventricular fibrillation. Despite the appearance of feeble activity, the drop in pH in cases in which the ventricles fibrillated was often as great as in cases in which they maintained a normally coördinated beat. Thus, in three cats samples were taken 9 minutes after arterial ligation and during this period the ventricles had maintained an apparently normal rhythm. The lowering of the pH of the coronary venous blood was 0.75, 0.79 and 0.79 in these cases, whereas in another animal the coronary venous blood showed a drop in pH of 0.81 after 3 minutes of normal rhythm followed by 3 minutes of ventricular fibrillation.

The rapid lowering of the pH of the coronary venous blood which followed ligation of the coronary arteries is particularly striking when one considers that the change in pH in the heart muscle must have been much greater. In order to obtain samples from the veins of the ischemic heart it was necessary to prevent their collapse by ligating them proximally immediately after ligating the arteries. Each venous sample consisted, therefore, of normally alkaline venous blood, trapped in the veins at the moment of ligation, admixed with acid blood added to the venous sample as the heart contracted, the first ingredient serving to dilute and to neutralize the second. The great lowering of pH, even to 6.6 and below, in such mixtures, reflected a considerably greater fall in pH in the capillaries and tissue spaces of the laboring muscle.

It has been demonstrated that when 1.5 cc. of an isotonic solution of pH 6.0 is injected down the femoral artery of the cat pain-endings are stimulated (Moore, Moore and Singleton, 1934). In such an experiment the acidity of the solution must be reduced greatly by the neutralizing substances which the agent encounters in its course down the arterial tree and through the capillary wall to the nerve-endings in the intercellular spaces. It is evident, then, that pain-endings are sensitive to a degree of acidity definitely less than that represented by pH 6.0. Certain of the results reported at this time indicate that within a few minutes after ligation of both coronary arteries there developed in the tissue spaces of the heart an acidity which exceeded that represented by 6.5. In these animals the experimental period was limited to a few minutes because of impending death of the animal from circulatory failure consequent to ventricular fibrillation. The individual muscle fibers were not exhausted but were still contracting at the time the venous blood samples were taken. In the clinical case of myocardial infarction, however, a less extensive area of the heart being involved, the organ as a whole often continues its function for many hours while the infarcted area becomes exhausted to the point of necrosis. The experimental results suggest that in such clinical cases the accumulation of acid metabolites in the involved muscle can well be of a magnitude to account for pain. A similar conclusion is suggested with reference to the pain in ischemic extremities, since

Katz, Kerridge and Long (1925) found that skeletal muscle becomes even more acid than cardiac muscle when stimulated to fatigue.

SUMMARY

Both coronary arterial trunks were ligated in anesthetized cats. The heart became pale and after a few minutes there were reflex signs of a stimulation of pain-elements. In many experiments ventricular fibrillation ensued. In every case the ventricles became widely dilated within ten minutes after ligation of the arteries. After a period of ischemia varying from 3 to 9 minutes a sample of blood was taken from a coronary vein and a control sample from the vena cava. The coronary venous blood showed a marked lowering of pH whereas its lactic-acid content was increased greatly. The actual fall in pH, as indicated by comparison with blood from the vena cava, varied from 0.22 to 0.81. It exceeded 0.50 in nine of eighteen cats. In three animals the pH of the coronary blood fell below 6.6 and in one animal it reached 6.40. These pH values, we believe the lowest ever reported for blood from the living animal, illustrate how rapidly acid metabolites accumulate when functioning tissues are isolated from the arterial circulation.

Identical experiments were performed upon five dogs with results similar to those obtained in the cat.

In a separate series of cats all the major cardiac veins were ligated to produce venous congestion. Although the heart became markedly congested and its surface "wept" from transudation, the lowering of the pH of the coronary venous blood was so slight as to be questionable and there was only a small change in the lactic-acid content. After twenty or thirty minutes of congestion the heart continued to perform its function in an apparently normal manner.

Under the conditions of the experiments the lowering of the pH of the coronary venous blood to 6.6 or below after a few minutes of ischemia indicates a much greater acid-change in the cardiac muscle. The significance of this chemical alteration is discussed with reference to the occurrence of pain in ischemic lesions of the heart and of the extremities. In view of the sensitivity of pain-endings to acid (Moore, Moore and Singleton, 1934), it is concluded that in such clinical cases the accumulation of acid metabolites in the area devoid of arterial circulation can well be of a magnitude to account for the pain.

We wish to express our indebtedness to Dr. B. M. Hendrix for his help and advice in carrying out this study.

REFERENCES

AVERY, B. F. AND A. B. HASTINGS. J. Biol. Chem. **94**: 273, 1931.
CULLEN, G. E. J. Biol. Chem. **83**: 535, 1929.

DAWSON, W. T. AND O. BODANSKY. Proc. Soc. Exper. Biol. and Med. **28**: 635, 1931.
GRAYZEL, D. M., R. TENNANT, S. W. STRINGER AND F. A. SUTHERLAND. Proc. Soc. Exper. Biol. and Med. **31**: 837, 1934.
GROSS, L. The blood supply to the heart. Paul B. Hoeber, New York, 1921.
HIMWICH, H. E., W. GOLDFARB AND L. H. NAHUM. This Journal **109**: 403, 1934.
KATZ, L. N. AND C. N. H. LONG. Proc. Roy. Soc. London **99B**: 8, 1925.
KATZ, L. N., P. T. KERRIDGE AND C. N. H. LONG. Proc. Roy. Soc. London **99B**: 26, 1925.
KOLTHOFF, I. M. The colorimetric and potentiometric determination of pH, p. 103. John Wiley and Sons, Inc., New York, 1931.
KUHL, A. Klin. Wchnschr. **13**: 1529, 1934.
MCGINTY, D. A. AND A. T. MILLER, JR. This Journal **103**: 712, 1933.
MENDEL, B. AND I. GOLDSCHEIDER. Biochem. Ztschr. **164**: 163, 1925.
MOORE, R. M., R. E. MOORE AND A. O. SINGLETON, JR. This Journal **107**: 594, 1934.

THE OXYGEN AND CARBON DIOXIDE CONTENT OF THE ARTERIAL AND VENOUS BLOOD OF NORMAL SUBJECTS[1]

J. M. LOONEY AND E. M. JELLINEK

From the Memorial Foundation for Neuro-Endocrine Research and the Research Service, Worcester State Hospital, Worcester, Mass.

Received for publication August 10, 1936

In the course of our investigations into the physiological findings in schizophrenic patients we have been confronted time after time with the lack of adequate data in the literature for the values of various blood constituents in normal individuals. Many of the values given in the text books of physiology are taken from investigations of subjects classed as "normal controls" but who in reality were patients in hospitals under treatment for conditions which were assumed not to influence the variable under consideration. The data reported as regards the oxygen and carbon dioxide content of arterial and venous blood of normal subjects show marked variation. The published mean values for venous oxygen given in table 1 vary from 15.6 volumes per cent to 10.67, and that for venous carbon dioxide from 57.51 to 49.20 volumes per cent. The values for arterial gases, though they do not show such marked variations, range for oxygen from 18.07 to 21.51 volumes per cent, and for carbon dioxide from 46.68 to 52.76. This results in the anomalous situation that the mean carbon dioxide content of arterial blood, as given by one investigator, is greater than that of the value for venous blood as given by another investigator.

Several years ago we began a series of investigations on the oxidative processes in schizophrenic patients and as one of the steps in that program determined the venous blood gases in a group of normal subjects. This material has been withheld from publication pending the determination of the gaseous content of the arterial blood of normal subjects. We have, at last, been able to utilize for this experiment a group of normal healthy male subjects who have been paid to live in the hospital and undergo a series of tests designed to establish normal values for many physiological variables.

In this study we have collected values for the venous blood gases of 67 normal subjects and for the arterial blood gases of 29 normal subjects.

[1] This investigation was aided by a grant from the Rockefeller Foundation.

All of these individuals were healthy males, ranging in age from 18 to 45 years in whom careful physical and laboratory examinations failed to show any evidence of disease. To our knowledge, this group has yielded the largest sample of analyses that has been presented on this subject and the only one in which the relationships among the various blood gases have been subjected to biometric analysis.

METHODS. The arterial and venous blood samples were obtained, one immediately after the other, in the morning while the subjects were in a basal state. The arterial puncture was made in the radial and brachial

TABLE 1

Blood gases in normal individuals

INVESTIGATOR	ARTERIAL		VENOUS		CASES
	O_2	CO_2	O_2	CO_2	
Henderson et al. (1924)	19.35	49.21	14.95	52.82	2
Doisy et al. (1922)			12.23	52.43	3
Florkin, Edward and Dill (1930)			14.62		10
Stadie, W. C. (1919)	20.2		15.6		5
Harrop, C. A. (1919)	19.41(17)*	49.69(10)	14.04(15)	54.65(10)	
Goldschmidt and Light (1925)			14.21	52.9	35
Lundsgaard and Moller (1923)			15.58	49.20	16
Barr, Himwich, and Green (1923)			10.67	55.30	2
	20.88	47.94			7
Cullen and Robinson (1923)			10.71	57.51	21
Burwell and Robinson (1924)	21.51(11)	46.68(8)			
Meakins and Davies (1920)	18.07(11)	52.76(7)			
Weiss and Ellis (1935)			12.9		17
Hurtado, Kaltreider and McCann (1934)	19.15	46.84			2
Weighted grand mean	19.80(56)	49.08(36)	13.60(126)	53.55(89)	
Looney and Jellinek	19.00(29)	49.9(29)	10.6(67)	57.8(67)	

* Figures in parenthesis designate number of cases.

arteries after preliminary anesthesia with novocain. The venipuncture was made in the median basilic vein. A tourniquet was used to facilitate the entrance of the needle into the vein and immediately released. In the earlier tests, thirty seconds were allowed to elapse before drawing the sample, whereas in the later studies a delay of one minute ensued before taking the blood. That these intervals were sufficient to prevent any significant changes as the result of stasis was shown by a study of Looney and Childs (1934). The blood was collected and stored in a 20 cc. syringe in which a suitable amount of a finely powdered mixture of sodium fluoride and potassium oxalate was placed to prevent coagulation. The details

of this method of collection and data showing its superiority to collections under oil are given in the above paper of Looney and Childs (1934). The analyses were made within a few hours by the manometric method of Van Slyke (1924). One of the advantages of the method that was used was that no change in the gaseous content of the sample occurred within the period of storage utilized.

RESULTS. For the arterial values we are restricted to the 29 cases determined during the comparative study, but for the venous values we have in addition the results of our analyses on 38 normal subjects from our previous study. As a careful statistical analysis[2] indicated that there were no significant differences between the two samples, they were merged for determination of the various functions and correlations. In table 2 are given the results of the analyses with the means, standard deviations and their standard errors. In this connection we wish to emphasize the fact that the values for the venous blood gases were obtained from two groups determined two years apart. The earlier group of 38 individuals

TABLE 2

Blood gases of normal subjects

	NUMBER OF CASES	MINIMUM	MAXIMUM	MEAN AND S.E.	STANDARD DEVIATION AND S.E.
		vol. per cent	*vol. per cent*	*vol. per cent*	*vol. per cent*
Arterial O_2	29	10.5	22.8	19.0±0.47	2.5±0.33
Arterial CO_2	29	40.8	57.4	49.9±0.51	2.7±0.36
Venous O_2	67	4.0	17.5	10.6±0.36	2.9±0.25
Venous CO_2	67	49.7	63.5	57.8±0.35	2.8±0.25

gave a value of 10.97 ± 0.47 volumes per cent for the oxygen and 54.43 ± 0.52 volumes per cent for carbon dioxide. These values are essentially the same as those for the later group, a fact which lends considerable evidence in support of their representativeness.

Our mean for venous oxygen, viz., 10.6 volumes per cent, is lower than that given by most investigators (approximately 14.00 volumes per cent) but Barr, Himwich and Green (1923) report a value of 10.67, and Cullen and Robinson (1923) one of 10.71. It is to be noted, moreover, that the figure obtained by Cullen and Robinson was from a group of 21 medical students and is therefore closely analogous to our own group of 67 controls; the essential identity of their and our values may be thus explained.

For the carbon dioxide content, a similar discrepancy is found though in a reverse direction. Here again the value obtained by Cullen and Robinson of 57.51 volumes per cent is in close agreement with our value of 57.8. The lowest mean value of 49.2 volumes per cent, was obtained

[2] The statistical evaluation of this material is considered in a separate report.

in 16 cases by Lundsgaard and Moller (1923) who also give the highest value for venous oxygen of 15.58 volumes per cent.

A possible explanation of the wide variations shown in table 1 would appear to lie in the fact that most investigators have relied upon oil to protect the blood after withdrawal from gaseous interchange. As Looney and Childs (1934) have shown, an average gain in oxygen of 1.32 volumes per cent and a loss of carbon dioxide of 1.86 volumes per cent can occur in less than two hours in bloods stored under oil. Considerable differences would arise in blood samples held under oil, depending on the time elapsing between taking the samples and analyzing them. This change would be more noticeable in venous than in arterial blood which had already been exposed to conditions approximating atmospheric in the lungs. A perusal of the arterial gas values given in the table lends further support to this conjecture. These values are notably more consistent than those of the venous blood and the grand means do not differ significantly from the means of our figures, although here again our oxygen value of 19.0 is somewhat lower than that of 19.8 taken from the collected figures, and our carbon dioxide value of 49.9 is correspondingly higher than the grand mean of 49.1 obtained from the literature. The findings of Meakins and Davis are questionable inasmuch as their mean of 52.8 for arterial carbon dioxide is equal to or higher than the venous carbon dioxide means of many of the investigators. Similarly their arterial oxygen mean of 18.07 is the lowest value recorded.

The mean of the difference between arterial and venous oxygen in our 29 normal subjects was 8.8 $\pm$ 0.59 volumes per cent with a minimum of 2.5 and a maximum of 14.5 volumes per cent. The corresponding value between venous and arterial carbon dioxide was 8.4 $\pm$ 0.63 with a range from 0.5 to 18.5 volumes per cent. This difference is greater than that of most of the values recorded in the literature chiefly because of the high venous oxygen and the low venous carbon dioxide values previously obtained.

We have also calculated the degree of oxygen saturation from the hemoglobin values obtained by the Haden hemoglobinometer.[3] For arterial blood we obtained a mean value of 91 per cent and for venous blood 48 per cent saturation. The differences between our values and those given in the literature are of the same magnitude as the variations in the oxygen content of the bloods.

The coefficients of correlation for the various blood gases have not previously been reported in the literature, probably because of the lack of data on a sufficiently large sample to render the results of value. In table 3 the results of these calculations for 29 arterial and 65 venous blood-gas determinations are recorded.

[3] O_2 = Hgb (gm./100 cc.) + 0.746

A study of this table shows that the level of venous oxygen is practically independent of the level of arterial oxygen; the level of the oxygen in venous blood is determined by the state of the tissues through which it flows and the rate of circulation. The tissues appear to be able to secure whatever oxygen they need regardless of the level of oxygen in the arteries, provided only that their oxygen content is above a certain minimal value approximating 12 volumes per cent. It was somewhat unexpected, however, to find that even less correlation exists between the arterial and venous values for carbon dioxide. Here the coefficient of correlation is 0.03 so that we are forced to conclude that in resting normal subjects the carbon dioxide content of the venous blood and its level in the arterial blood are determined by essentially independent factors. Any common factors would necessarily disclose their operation in a correlation coefficient of positive sign. A reciprocal relationship would be evidenced by a coefficient of significant magnitude but of negative sign.

TABLE 3

Coefficients of correlation on regression equations in normal subjects

	N	*r*	REGRESSION EQUATION
Arterial O_2 vs. venous O_2	29	0.30	
Arterial O_2 (x) vs. arterial CO_2 (y)	29	−0.54	Y = 61.4 − 0.60 x
Arterial CO_2 vs. venous CO_2	29	0.03	
Venous O_2 (x) vs. venous CO_2 (y)	65	−0.76	Y = 65.4 − 0.72 x

Since the body uses only a relatively small fraction of the total buffering substances of the blood to combine with the carbon dioxide produced in the tissues during rest, it would seem likely that in the basal state a relatively high correlation should exist between the arterial and venous levels of carbon dioxide. This would be brought about by the large percentage of carbon dioxide-combining materials circulating unchanged in both systems. The facts seem to indicate, however, that the governing factor is not in any significant degree the level of carbon dioxide in the arterial blood but practically entirely the length of exposure of the blood to the tissues.

A fair degree of correlation exists between arterial oxygen and carbon dioxide, a negative correlation of −0.54 being obtained, and a still higher correlation was found between venous oxygen and carbon dioxide with a correlation coefficient of −0.76. These correlations are lower than might have been expected in view of the physico-chemical relationships existing between carbon dioxide and oxygen in blood as set forth by Henderson (1928).

Since 85 per cent of the buffering substances of the blood used to neu-

tralize the carbonic acid produced in the tissues are due to the fixed bases released in the red blood corpuscles during the change in acidity caused by the reduction of oxyhemoglobin to reduced hemoglobin, it would appear reasonable to look for a close relationship between the oxygen and carbon dioxide values. As indicated by the correlation coefficients, actually only 36 per cent of the variation in venous carbon dioxide can be accounted for by the variation in venous oxygen, and only 16 per cent of the variation in arterial carbon dioxide can be accounted for by the variation in venous oxygen. The relationships undoubtedly would be much closer for variations of the gases taken repeatedly in the same individual, but our samples taken two weeks apart are too small to give satisfactory data for this purpose. We have used these results, however, to obtain a measure of self-correlation of the blood gases in terms of intra-class correlation for the 29 normal subjects. The results are as follows: arterial oxygen, 0.37; venous oxygen, 0.38; arterial carbon dioxide, 0.01; and venous carbon dioxide, 0.22. The values are all very low and indicate that the basal level of blood gases within the individual is extremely variable. This variation was also shown in our past studies in which the samples were taken three months apart. A search of the literature has failed to reveal any very precise data on this point, although in various investigations several determinations have been made on the same subject. In the study of Barr, Himwich, and Green (1923) repeated analyses of the venous blood of one subject gave oxygen values varying from 4.5 to 16.2 volumes per cent and a carbon dioxide content ranging from 49.2 to 59.9 volumes per cent. In another subject the arterial oxygen content varied from 17.55 to 20.0 volumes per cent while the carbon dioxide content varied from 50.45 to 54.5 volumes per cent.

It is evident that a single determination of the blood gases is insufficient to characterize the gaseous content of either the arterial or venous blood of normal subjects.

SUMMARY. The carbon dioxide and oxygen contents were determined in the arterial blood of 29 and in the venous blood of 67 normal men.

The mean value for the venous oxygen was 10.6 $\pm$ 0.36 volumes per cent and 85 per cent of the readings fell between 7.0 and 14.0 volumes per cent. The mean value for carbon dioxide was 57.8 $\pm$ 0.35 volumes per cent.

These values were found to be significantly different from the mean of the mean values recorded in the literature, namely, 13.60 volumes per cent for oxygen and 53.55 volumes per cent for carbon dioxide.

The values for the arterial blood gases—19.0 $\pm$ 0.47 for oxygen and 49.9 $\pm$ 0.51 for carbon dioxide—did not differ significantly from the mean of the mean values reported previously.

There was a marked variability in the level of the blood gases in the

same individual as measured by the self-correlation in terms of intra-class correlation coefficients; these values were quite low ranging from 0.38 for venous oxygen to 0.01 for arterial carbon dioxide.

No significant correlation was found between the levels of arterial and venous oxygen or carbon dioxide. A fair degree of correlation was given between arterial oxygen and carbon dioxide, with a coefficient of -0.54; and a slightly higher correlation occurred between these gases on the venous side, with a coefficient of -0.76.

CONCLUSIONS

The normal mean value of venous oxygen is lower, and the mean value of venous carbon dioxide is higher than the values commonly accepted. These differences are probably due to reliance on oil to protect the blood gases from exchange with the atmosphere.

The levels of these gases in both arterial and venous bloods show considerable variation in the same individual at different times hence a single determination cannot be relied on to characterize the blood gases.

REFERENCES

BARR, D. P., H. E. HIMWICH AND R. P. GREEN. J. Biol. Chem. **55**: 495, 1923.

BURWELL, C. S. AND C. C. ROBINSON. J. Clin. Invest. **1**: 87, 1924.

CULLEN, G. E. AND H. W. ROBINSON. J. Biol. Chem. **57**: 533, 1923.

DOISY, E. A., A. P. BRIGGS, E. P. EATON AND W. H. CHAMBERS. J. Biol. Chem. **54**: 305, 1922.

FLORKIN, M., H. T. EDWARD AND D. B. DILL. This Journal **94**: 460, 1930.

GOLDSCHMIDT, S. AND A. B. LIGHT. This Journal **73**: 127, 1925.

HARROP, C. A. J. Exper. Med. **30**: 241, 1919.

HENDERSON, L. J., A. V. BOCK, H. FIELD, JR. AND J. L. STODDARD. J. Biol. Chem. **59**: 379, 1924.

HENDERSON, L. J. Blood: A study in general physiology. Yale University Press, 1928.

HURTADO, A., N. KALTREIDER AND W. S. MCCANN. This Journal **109**: 626, 1934.

LOONEY, J. M. AND H. CHILDS. J. Biol. Chem. **104**: 53, 1934.

LUNDSGAARD, C. AND E. MOLLER. J. Biol. Chem. **55**: 477, 1923.

MEAKINS, J. AND H. W. DAVIES. J. Path. and Bact. **23**: 451, 1920.

STADIE, W. C. J. Exper. Med. **30**: 215, 1919.

VAN SLYKE, D. D. AND J. M. NEILL. J. Biol. Chem. **61**: 523, 1924.

WEISS, S. AND L. B. ELLIS. Arch. Int. Med. **55**: 665, 1935.

CARBOHYDRATE AND PHOSPHORUS CHANGES IN PROLONGED MUSCULAR CONTRACTIONS

JACOB SACKS, WILMA C. SACKS AND J. RAYMOND SHAW

From the Laboratory of Pharmacology, University of Michigan Medical School, Ann Arbor

Received for publication August 11, 1936

Studies on the "steady state" in muscular activity have been limited, for the most part, to investigation of changes in the blood and in the respiratory exchange. It has been assumed that these changes reflected accurately the conditions in the muscles themselves. It is necessary, however, to study the changes actually taking place in the muscles as they pass from the resting condition through the intermediate phases into the steady state of activity. The technique used in previous investigations on the chemistry of muscular contraction (6, 7) has been modified slightly in order to make these observations.

The change consisted of the substitution of a series of maximal isometric twitches at constant rate for the tetanic contraction used in the previous investigations. In this way the actual conditions of muscular work in man have been more closely approximated. The gastrocnemius muscles of cats under pentobarbital anesthesia were used. The muscles were dissected free except at the origin, and the blood supply and innervation were kept intact, as in all the previous work. One muscle served as the resting control, the other was stimulated. The muscle to be stimulated was attached to an isometric lever, put under tension of 200 to 400 grams,[1] and stimulated with maximal induction shocks at the desired rate. Immediately at the end of the stimulation period it was frozen *in situ* with a mixture of powdered carbon dioxide and ether; the resting muscle was then frozen in the same way.

It has been found in previous work by one of us (5) that the glycogen contents of right and left gastrocnemius muscles of a single cat might differ by 10 per cent or more. However, this muscle has such a high capacity for maintaining tension that it was felt to be a desirable one to use in spite of this possible difference, especially since this difference does not exist with respect to the content of the various phosphorus

[1] Maintaining the muscle under this tension for several minutes without stimulation does not produce any detectable change in any of the substances determined in these experiments.

compounds in the resting muscle. The technical details of the apparatus required that the right muscle be chosen for stimulation. Since the greater glycogen content might be present initially in either right or left muscle, the average figures for a group of animals would tend to minimize this variation.

Two different levels of activity were studied: in one series of experiments the stimulation rate was 1 per second, in the other, 2 per second. The two rates were selected arbitrarily, but the results obtained proved the choice to have been a happy one. The duration of stimulation was 1, 2, 5, or 10 minutes; in addition, some muscles were stimulated for 30 minutes at the higher rate.

The analytical procedures were those used in the previous studies (6, 7). The following phosphorus fractions were determined: (A) inorganic phosphate; (B) the sum of inorganic and phosphocreatine phosphorus; (C) the readily hydrolyzable phosphorus, i.e., the value obtained after 15 minutes' hydrolysis of the muscle filtrate with 1 N sulfuric acid at the temperature of boiling water; and (D) the total acid-soluble phosphorus. These fractions and lactic acid were all determined on aliquots from a single trichloracetic acid filtrate of the muscle. Glycogen was determined on a separate portion of the muscle, the sample being taken after the entire muscle had been ground to a powder while in the frozen state.

It was found that the muscles absorbed an appreciable amount of water from the blood stream during the stimulation period. This led to the necessity of correcting the directly determined analytical values. This was done by multiplying all the other values obtained on the stimulated muscle by the ratio between the total acid-soluble phosphorus values for the resting and stimulated muscles. This procedure is admittedly open to criticism on theoretical grounds, but there is no other feasible way by which the correction can be made with any greater accuracy. Dry weight determinations, which are much more tedious, lead to practically the same correction factor.

When the values for the muscles stimulated at the lower rate were corrected in this way, no further calculations were necessary. However, in the muscles stimulated twice per second, a considerable portion of the adenosine triphosphate was found to have undergone hydrolysis into adenylic (or inosinic) acid and two molecules of inorganic phosphate. This necessitated further calculation because the increase in inorganic phosphate in these muscles was derived from two sources: hydrolysis of phosphocreatine and hydrolysis of adenosine triphosphate. The amount of this latter compound present was determined from the difference between the C and B values for the muscle. The increase in directly determinable phosphate after acid hydrolysis for 15 minutes represents the two readily hydrolyzable phosphate groups of the nucleotide. Then, the

difference in the (C-B) values for the resting and stimulated muscles measures the amount of the nucleotide which has been hydrolyzed during the stimulation period. To determine the amount of phosphocreatine which has been hydrolyzed, the difference of the A values of the stimulated and resting muscles is taken, and from this difference is substracted the value for the inorganic phosphorus derived from the hydrolysis of adenosine triphosphate. The amount of hexosephosphate formed is obtained by subtracting the C value for the stimulated muscle from that for the resting one. To illustrate the calculation necessary for these experiments, the data for one animal and the calculations made therefrom are shown below. The muscle had been stimulated for two minutes. The figures are in terms of milligrams per cent of phosphorus.

	I RESTING	II STIMULATED	III STIMULATED, CORRECTED
A. Inorganic	19	74	85
B. Inorganic plus phosphocreatine	92	83	95
C. Acid hydrolysis	131	104	119
D. Total acid-soluble	156	136	(156)

The values in column III were obtained by multiplying those actually determined for the stimulated muscle (II) by 156/136, as stated above. Now the difference between the C and B values for a single muscle represents the two readily hydrolyzable phosphate groups of the nucleotide. The resting muscle thus contained 131 − 92, or 39 mgm. per cent of such phosphorus. The stimulated one contained 119 − 95, or 24 mgm. per cent. The amount which had undergone hydrolysis during the stimulation period was 39 − 24, or 15 mgm. per cent. Of the 85 mgm. per cent of inorganic phosphorus found in the stimulated muscle, 19 mgm. per cent had been present as such in the resting state. This 19, and the 15 mgm. per cent derived from hydrolysis of the nucleotide, must be subtracted from 85, giving 51 mgm. per cent of inorganic phosphorus formed by hydrolysis of phosphocreatine. As for the hexosephosphate formed: the phosphorus in it, now difficulty hydrolyzable, came from the readily hydrolyzed portion. Therefore 131 − 119, or 12 mgm. per cent, underwent this conversion.

Records of the tension developed were made on a kymograph drum. The initial contractions developed a definitely higher tension than the subsequent ones, but before the end of the second minute the tension per twitch had reached a practically constant level. The muscle was then considered to have reached the steady state. In some cases the twitch tension increased slightly after 5 or 6 minutes; however, the increase was small.

RESULTS. *Twitches at rate of 1 per second.* It can be seen from Table 1 that there is no formation of hexosephosphate nor hydrolysis of adenosine

TABLE 1

Changes in phosphorus compounds and lactic acid, and in carbohydrate and water content of gastrocnemius muscle of cats stimulated isometrically at rate of one twitch per second

Values are expressed as milligrams per cent of P and of lactic acid. Carbohydrate changes calculated as milligrams per cent of glucose equivalent.

PHOSPHOCREATINE HYDROLYZED	HEXOSEPHOSPHATE FORMED	ADENOSINE TRIPHOSPHATE HYDROLYZED	INCREASE IN LACTIC ACID CONTENT	CARBOHYDRATE BALANCE	WATER UPTAKE
a. Stimulation period one minute					
					per cent
16	0	0	29	+29	0
28	0	0	70	+10	1
22	0	0	61	+6	9
21	0	0	57	−183	1
24	9	0	91	−12	0
Av. 22	2	0	62	−30	2
b. Stimulation period two minutes					
37	0	0	49	−31	4
43	0	0	66	−68	6
42	0	0	80	−105	10
33	0	0	142	−98	4
30	0	0	49	−51	10
37	3	2	170	−88	11
Av. 37	0.5	0.3	93	−74	8
c. Stimulation period five minutes					
30	3	2	103	−123	7
32	3	7	126	−79	15
41	0	0	140	+25	11
55	0	0	208	−122	14
36	0	0	107	−86	11
Av. 39	1	2	137	−77	12
d. Stimulation period ten minutes					
35	0	0	109	−151	10
35	0	3	85	−157	17
25	0	0	90	−20	12
31	0	0	83	−48	14
14	3	−3	32	−82	3
Av. 28	0.6	0	80	−92	11

triphosphate at this rate of contraction, either in reaching the steady state from rest, or in maintaining the steady state. In the entire group of 21

experiments, only one shows hexosephosphate formation greater in amount than probable experimental error, and only one shows definite hydrolysis of adenosine triphosphate. It can thus be stated that at the level of muscular activity represented by this rate of stimulation, neither of these two reactions is involved.

The lactic acid content shows the greatest increase during the first minute. During the second minute a smaller amount of lactic acid is formed, and in the succeeding minutes even smaller amounts; after 5 minutes the loss by diffusion and oxidation exceeds the formation. The data on carbohydrate balance are incomplete in that muscle glucose was not determined, but the amount of glucose formed in the muscle during stimulation is probably too small to affect the findings. During the steady state there is a progressive loss of carbohydrate from the stimulated muscle. This loss apparently consists of lactic acid oxidized and also of lactic acid lost to the muscle by diffusion into the blood stream.

The amount of phosphocreatine hydrolyzed tends to be parallel to changes in lactic acid content, increasing as lactic acid accumulates in the muscle, and being resynthesized from its hydrolysis products as lactic acid is removed from the muscle.

Twitches at rate of 2 per second. Examination of table 2 shows that this rate of stimulation leads to an entirely different picture, so far as phosphate changes are concerned. The most striking difference is the hydrolysis of adenosine triphosphate. This begins during the first minute, about one-third of that originally present being hydrolyzed. Throughout the steady state this hydrolysis continues to take place, but at a much slower rate.

A second difference from the findings at the lower stimulation rate is in the behavior of hexosephosphate. During the first minute of stimulation a large amount of this substance is formed; thereafter the total amount present decreases. In the steady state, as at the lower level of stimulation, this compound is not formed, and that which accumulated during the anaerobic period is removed as the steady state continues.

The lactic acid content reaches a very high level at the end of the first minute; the concentration remains practically constant for ten minutes, and only after this time does it begin to decrease. It is further seen that more than three times as much lactic acid is formed in 120 twitches in one minute than in 60 twitches in one minute. The total loss of carbohydrate at this stimulation rate is a little more than twice that at the lower level of stimulation.

Water absorption begins early and reaches a maximum shortly after the steady state is reached. Greater absorption of water takes place at the higher stimulation rate.

TABLE 2

Changes in phosphorus compounds and lactic acid and in carbohydrate and water content of gastrocnemius muscles of cats stimulated isometrically at rate of two twitches per second

Values are expressed as milligrams per cent of P and of lactic acid. Carbohydrate changes calculated as milligrams per cent of glucose equivalent.

	PHOSPHOCREATINE HYDROLYZED	HEXOSEPHOSPHATE FORMED	ADENOSINE TRIPHOSPHATE HYDROLYZED	INCREASE IN LACTIC ACID CONTENT	CARBOHYDRATE BALANCE	WATER UPTAKE
	a. Stimulation period one minute					
						per cent
	30	11	6	164	+98	3
	46	11	0	221	−20	11
	33	7	2	115	−99	11
	31	7	9	152	−213	0
	27	19	15	312	−128	13
	25	33	25	343	+129	15
Av.	32	15	10	218	−39	9
	b. Stimulation period two minutes					
	53	7	6	191	−8	11
	51	12	15	228	−109	12
	40	14	10	284	−50	10
	42	16	15	319	−103	13
	42	2	−1	126	−283	4
	54	7	9	207	+57	4
	38	12	12	284	−111	12
Av.	46	10	9	234	−87	9
	c. Stimulation period five minutes					
	30	7	5	145	−89	11
	39	1	0	121	−108	7
	48	0	20	283	−262	36
	43	3	20	209	−227	25
	59	4	10	236	−1	22
	54	4	14	319	+17	22
	42	1	18	196	−152	20
Av.	45	3	12	215	−117	20
	d. Stimulation period ten minutes					
	35	0	4	124	−306	17
	37	2	6	120	−190	16
	41	2	7	79	−179	12
	54	−3	20	293	−272	21
	57	2	19	303	−276	29
	54	0	18	284	−227	33
	52	3	18	280	−281	33
	45	1	17	336	−322	14
Av.	46	1	14	227	−257	22

TABLE 2—*Concluded*

PHOSPHOCREATINE HYDROLYZED	HEXOSEPHOSPHATE FORMED	ADENOSINE TRIPHOSPHATE HYDROLYZED	INCREASE IN LACTIC ACID CONTENT	CARBOHYDRATE BALANCE	WATER UPTAKE
e. Stimulation period thirty minutes					
43	−2	15	98	−690	18
39	0	18	98	−503	18
9	3	6	71	−462	23
38	5	24	152	−438	27
32	1	17	96	−779	15
Av. 32	1	16	103	−574	20

Discussion. The results may conveniently be discussed with respect to the changes undergone by each of the substances involved, in the approach to, and maintaining of, the steady state.

Lactic acid. In the early stages, before the steady state is reached, there is a large formation of this substance. This is relatively greater at the higher stimulation rate. During the steady state there is a continuous formation of lactic acid, varying in amount with the degree of activity. The quantity formed, however, is quite small in comparison with that formed during the early part of the work period. This continuous formation may be due to local areas of oxygen deficiency, in which contraction is accomplished by anaerobic reactions, or it may be another example of the aerobic glycolysis seen in other tissues with high metabolic rates.

The figures for carbohydrate balance, taken in conjunction with those for lactic acid content, support the view previously expressed (7) that lactic acid is not converted to glycogen, except possibly in traces, within the muscle itself. It has been shown that the recovering muscle is unable to accomplish this synthesis; it is hardly to be expected that the working one would be able to bring about a "recovery" change of which the muscle actually recovering is incapable.

Phosphocreatine. The hydrolysis and resynthesis of this substance are parallel to changes in lactic acid concentration, as would be expected; the buffer substance is formed or removed as the amount of acid to be buffered increases or decreases.

Hexosephosphate. The formation of this substance from phosphocreatine and glycogen evidently follows a different course from the hydrolysis of phosphocreatine. The amount formed is related to the intensity of the anaerobic activity undergone by the muscle. At a low level of activity, the substance is not formed; at the higher level, it is formed only during the period of oxygen deficiency. During the steady state it is resynthesized to phosphocreatine. Since the formation of this substance furnishes both energy and buffer for lactic acid, it would appear that the

reaction is in the nature of an "anaerobic reserve," called upon only when the glycolytic mechanism is quantitatively inadequate.

Adenosine triphosphate. In the schemata of Lohmann (2) and of Parnas and his co-workers (4), the initial reaction in contraction is considered to be the breakdown of this substance to adenylic acid and orthophosphate or hexosediphosphate. However, these formulations were derived from studies of enzyme reactions in cell-free muscle extracts. Considerable caution must be exercised in applying these conclusions to intact muscle undergoing contraction, especially since the substance does not undergo any decomposition either in tetanus (6) or in twitches at the rate of one per second. Only when the rate of stimulation is raised to two per second does any decomposition of adenosine triphosphate become evident. The change is most marked during the period of anaerobic activity, and is greatest in those cases in which the lactic acid accumulation is highest. This last finding is contrary to what would be expected from the formulations of Lohmann and Parnas, according to which adenosine triphosphate is resynthesized by energy obtained ultimately from the formation of lactic acid.

Breakdown of adenosine triphosphate with the formation of orthophosphate has been reported by Lundsgaard (3) in muscles poisoned with iodoacetic acid and contracting in nitrogen, and by Embden, Hefter, and Lehnartz (1) in fatigued frog muscles. It is apparent that only under adverse conditions, such as those mentioned, does this substance undergo any breakdown. The question may therefore well be raised, whether the decomposition of adenosine triphosphate during contraction is not a manifestation of cell injury rather than a vital link in the orderly chain of reactions which normally yield the energy for muscular activity.

SUMMARY AND CONCLUSIONS

1. Changes in glycogen, lactic acid, and the phosphorus compounds have been studied in the muscles of cats subjected to prolonged stimulation *in situ* at the rates of 1 and 2 twitches per second.

2. The "steady state" is reached by these muscles within two minutes.

3. At the lower stimulation rate there is no formation of hexosephosphate nor hydrolysis of adenosine triphosphate; at the higher rate both these changes are found to take place before the steady state is reached, and the latter continues during the steady state.

4. During the steady state any hexosephosphate previously accumulated in the muscle tends to be removed.

5. More than three times as much lactic acid is formed in the first minute at 2 twitches per second than at 1 twitch per second.

6. Phosphocreatine undergoes hydrolysis when lactic acid is accumulating, and is resynthesized when lactic acid is lost from the muscle.

7. The steady state is characterized by a continuous formation of lactic acid in small amount; the rate of this continuous formation depends on the intensity of activity of the muscle.

8. The working muscles absorb large amounts of water from the blood stream.

REFERENCES

(1) EMBDEN, G., J. HEFTER AND M. LEHNARTZ. Hoppe-Zeyler's Ztschr. **187**: 53, 1930.
(2) LOHMANN, K. Biochem. Ztschr. **271**: 264, 1934.
(3) LUNDSGAARD, E. Ibid. **269**: 308, 1934.
(4) PARNAS, J. K., P. OSTERN AND T. MANN. Ibid. **275**: 74, 1934.
(5) SACKS, J. This Journal **97**: 467, 1931.
(6) SACKS, J. AND W. C. SACKS. This Journal **105**: 151, 1933.
(7) SACKS, J. AND W. C. SACKS. This Journal **112**: 565, 1935.

PITUITRIN ANEMIA

ALFRED GILMAN AND LOUIS GOODMAN

From the Department of Pharmacology and Toxicology, Yale University, School of Medicine, New Haven, Connecticut

Received for publication August 15, 1936

In recent publications Dodds and co-workers (1, 2) reported a severe anemia in rabbits after the administration of subcutaneous pituitrin. This anemia occurred on the fourth to fifth day after pituitrin and was characterized by macrocytosis, hyperchromia, marked reticulocytosis and leucocytosis, and an increase of bile in the intestine. They concluded that "the control of blood destruction by the reticulo-endothelial system may be vested outside the system and may reside in the posterior lobe of the pituitary gland." Although a number of the constituents of the blood plasma are known to be under hormonal regulation, this was the first suggestion that the cellular elements of the blood may be influenced by hypophyseal activity.

In order to produce this anemia, Dodds injected a massive dose of pituitrin amounting to 200 pressor units per kilogram of body weight. It does not seem reasonable, however, to attribute to a gland a physiological rôle on the basis of evidence obtained from the injection of a pharmacological preparation of that gland in such large amount.

Before attributing a new function to the posterior hypophysis the known pharmacological actions of pituitrin should be examined for their ability to produce such striking effects on the blood. The well-known antidiuretic action of pituitrin, resulting in body hydration and a decrease in the osmotic pressure of the fluid environment of the red corpuscles, immediately suggests itself as the most likely explanation. The fact that the blood picture described above characterizes several experimentally produced hemolytic anemias strengthens the explanation that the anemia results from intravascular hemolysis in hypotonic serum due to pituitrin-induced water retention.

METHODS AND PROCEDURES. Healthy adult rabbits were used in all experiments. They were kept in individual metabolism cages, and weighed daily. Quantitative collections of urine were made, and the specific gravity recorded. The basic diet was one of high water content and consisted of cabbage, lettuce, and carrots ad libitum. In dehydration experiments oats only were allowed.

The following blood data were obtained at frequent intervals: hemoglobin percentage, red and white cell counts, hematocrit reading, reticulocyte percentage, fragility to hypotonic saline, serum osmotic pressure (Hill, 3), serum specific gravity (Barbour and Hamilton, 4), and icteric index. For the last four determinations, 3 cc. of blood were obtained by cardiac puncture; otherwise, blood from the marginal ear vein was used.

After a five day preliminary observation period during which two control blood determinations were made, the animals were injected subcutaneously with pituitrin[1] in the dosage of 200 units per kilogram. Normal rabbits, kept under identical conditions but not given pituitrin, served as controls.

RESULTS. Thirty-one experiments were performed on twenty-six rabbits. The average urine and blood changes in those animals subjected to the standard experimental procedure, namely, a wet diet and a single 200 unit/kilo dosage of pituitrin, are summarized in table 1. A more detailed analysis of the results follows.

Food intake. Following the injection there was a marked reduction in the food intake of the rabbits. This varied considerably, some animals refusing all food, others consuming 25 to 75 per cent of their preinjection daily intake. Normal appetite returned about one week after the pituitrin administration.

Body weight. The body weight of the animals was largely a reflection of the food intake. However, due to the marked pituitrin-induced water retention even in those rabbits which stopped eating the weight losses were slight, amounting in the majority of instances to 100–200 grams. In those animals which continued to eat there was a gain. In one instance this amounted to 500 grams (25 per cent of the body weight) in the six days following injection. This gain in weight was obviously due to water retention as indicated by 1, the accompanying oliguria; 2, the edema of the subcutaneous tissue; 3, the marked hydration of the body fluids as evidenced by the values for blood osmotic pressure and specific gravity, and 4, the rapid return of the body weight to normal seven days after the pituitrin administration coincident with a marked diuresis.

Urine volume. The urine volume of the rabbits on a wet diet averaged 500 cc. daily. This was markedly reduced as a result of the pituitrin injection. The effects were noticed immediately and were most marked 48 to 72 hours after administration. Many of the animals were anuric over this period. Normal renal function was again established seven days after injection.

Urine specific gravity. The specific gravity of the urine varied inversely with the volume. As would be expected, after the administration of pituitrin, the kidney was capable of concentrating urine to a high degree.

[1] Kindly supplied by Dr. Oliver Kamm, of the Parke-Davis Company.

Serum osmotic pressure. The pituitrin-induced water retention was clearly reflected in the osmotic pressure of the serum. The normal values for serum osmotic pressure were somewhat low, probably as a result of the high water content of the diet. The average serum of the animals before injection was isosmolar with 0.866 per cent NaCl. Following pituitrin injection this value fell markedly and usually reached its lowest on the fifth day. This corresponds to the time at which Dodds observed severe anemia in his animals. The degree of blood dilution as evidenced by the serum osmotic pressure was fairly constant in all the animals. The average serum was isosmolar with 0.642 per cent NaCl, varying between

TABLE 1

Average urine and blood changes in rabbits following 200 units per kilo of subcutaneous pituitrin*

	NORMALS	POST-INJECTION VALUE	PER CENT CHANGE	MAXIMUM CHANGE NOTED ON:
				day
Urine volume, 24. hr.	500 cc.	35 cc	−93	3
Urine specific gravity	1.011	1.029	+164	3
Serum osmotic pressure†	0.866%	0.642%	−26	5
Serum specific gravity	1.0221	1.0178	−20	4
Red blood corpuscles	5.39 mil.	3.19 mil.	−40	7
Hemoglobin	12.0 gm.	8.0 gm.	−33	8
Hematocrit	34.0%	20.0%	−40	7
Fragility				
Hemolysis begins	0.50% NaCl	0.42% NaCl	16	
Hemolysis complete	0.40% NaCl	0.27% NaCl	33	

* Figures representing the maximal changes observed in each experiment are averaged in this table.

† Expressed in this and subsequent tables as percentage concentration of an isosmolar NaCl solution.

the extremes of 0.561 and 0.696 per cent. Thus it is obvious that the cellular constituents of the body were in an extremely unfavorable environment with respect to the osmotic pressure of their surrounding fluid media with which they must maintain equilibrium. The serum osmotic pressure remained low for several days, started to rise slowly coincident with the return of normal renal function and approached a normal figure ten to fourteen days after the pituitrin injection.

Serum specific gravity. The variations in specific gravity of the serum paralleled the serum osmotic pressure findings. Serum specific gravity is largely a reflection of the concentration of serum proteins. Thus a fall in specific gravity is evidence of a blood dilution due to water retention.

The fall observed indicates that increased excretion of electrolyte played little part in the reduced serum osmotic pressure. A low serum electrolyte concentration resulting from electrolyte excretion rather than water retention is characterized by an extreme degree of anhydremia (5, 6). Serum specific gravity returned toward normal coincident with the onset of diuresis and the recovery of serum osmotic pressure.

Red blood corpuscles. In every experiment there was evidence of an anemia. However, there were two types of responses observed. The first is illustrated in table 2. It is characterized by a marked reduction in cell count to a level slightly over one million. The onset of the anemia coincided with the fall in osmotic pressure of the serum. The anemia persisted as long as the osmotic pressure remained low, and the count

TABLE 2

Marked hemolytic anemia in a rabbit following the subcutaneous injection of 200 units per kilo of pituitrin

	PRE-INJECTION VALUE	MAXIMUM CHANGE TO:	3 WEEKS AFTER INJECTION
Body weight	1.92 kgm.	1.52 kgm.	1.66 kgm.
Urine volume, 24 hr.	500 cc.	40 cc.	500 cc.
Urine specific gravity	1.010	1.020	1.010
Serum osmotic pressure	0.880%	0.647%	0.868%
Serum specific gravity	1.0218	1.0172	1.0206
Red blood corpuscles	6.25 mil.	1.38 mil.	3.16 mil.
Hemoglobin	13.5 gm.-%	4.5 gm.-%	7.5 gm.-%
Hematocrit	34.5%	11.0%	25.0%
Fragility			
Hemolysis begins	0.52% NaCl	0.46% NaCl	
Hemolysis complete	0.36% NaCl	0.28% NaCl	
Reticulocytes	0.3%	49.7%	6.1%
Icteric index	6	10	6

slowly returned with the restoration of normal renal function. In this type of response it is obvious that there has been an actual destruction of a large number of cells. There occurred a prompt and high reticulocytosis, a temporary neutrophilic leucocytosis, a slight increase in icterus index, occasional slight hemoglobinuria, and marked changes in the appearance of the blood smear characteristic of rapid blood destruction and regeneration. Inasmuch as the return to a normal blood picture depends not only upon the restoration of normal water metabolism but also on the formation of new cells, it is not surprising to note that the anemia persisted after the osmotic pressure of the blood returned to normal.

In contrast to this severe anemia is the type depicted in table 3. Here the decrease in the number of R.B.C. was much less pronounced and

can be explained by blood dilution resulting in an increased circulating plasma volume, and a relative decrease of cellular elements. In this dilution anemia, inasmuch as there was little if any cell destruction, both the osmotic pressure and the cell count returned to normal following the release of the pituitary antidiuresis.

Two explanations may be offered to account for the above anemia: 1, that of Dodds which postulates a principle in the pituitary gland concerned with the destruction and formation of R.B.C., or 2, the authors' belief that the anemia is merely a result of a hemolysis initiated by the abnormal concentration of serum electrolytes resulting from the pituitrin-induced antidiuresis. The close time relationship between the changes in serum osmotic pressure and cell count, both with respect to onset and

TABLE 3

Dilution anemia in a rabbit following the subcutaneous injection of 200 units per kilo of pituitrin

	PRE-INJECTION VALUE	MAXIMUM CHANGE TO:	3 WEEKS AFTER INJECTION
Body weight	1.90 kgm.	2.00 kgm.	1.90 kgm.
Urine volume, 24 hr.	455 cc.	125 cc.	450 cc.
Urine specific gravity	1.013	1.025	1.010
Serum osmotic pressure	0.879%	0.696%	0.838%
Serum specific gravity	1.0231	1.0193	1.0228
Red blood corpuscles	4.90 mil.	4.16 mil.	4.96 mil.
Hemoglobin	12.5 gm.-%	10.5 gm.-%	10.5 gm.-%
Hematocrit	34.0%	28.0%	31.0%
Fragility			
Hemolysis begins	0.50% NaCl	0.44% NaCl	
Hemolysis complete	0.40% NaCl	0.26% NaCl	
Reticulocytes	0.8%	6.1%	1.1%
Icteric index	5	5	5

recovery of the anemia supports the second viewpoint. More positive proof, however, would be obtained if one could prevent the anemia following pituitrin injection by procedures which did not allow blood dilution. Following are two types of such experiments.

Animals were placed on an oats diet with water ad libitum for one week to allow for intestinal absorption and renal excretion of residual fluid from the previous wet vegetable diet. They were then dehydrated for two days and the blood picture ascertained. Following this they were injected with the usual dose of pituitrin, replaced on the oats diet, and allowed water in the belief that they would drink no more than necessary to return their osmotic pressure to normal. Obviously with the removal of all excess water from the gastrointestinal tract by the previous dietetic

regimen, antidiuresis could not result in any marked blood dilution. The results are depicted in table 4. It can be seen that dehydration resulted in a high serum osmotic pressure and cell count. Following pituitrin injection and access to water the animals consumed merely sufficient fluid to restore their blood chemistry to normal. As a result, the cell count returned to normal, and there was no indication of anemia.

TABLE 4

The prevention of pituitrin anemia by dehydration prior to injection

	PRE-INJECTION DEHYDRATION VALUE	MAXIMUM CHANGE AFTER PITUITRIN TO:
Body weight	1.84 kgm.	1.80 kgm.
Serum osmotic pressure	1.086%	0.905%
Serum specific gravity	1.0258	1.0239
Red blood corpuscles	7.00 mil.	5.46 mil.
Hemoglobin	15.0 gm.-%	12.5 gm.-%
Hematocrit	37.0%	31.0%
Fragility		
Hemolysis begins	0.59% NaCl	0.49% NaCl
Hemolysis complete	0.41% NaCl	0.34% NaCl
Reticulocytes	1.5%	3.3%
Icteric index	6	5

TABLE 5

The prevention of pituitrin anemia by preliminary alimentary dehydration, and a limited water intake subsequent to injection

	PRE-INJECTION VALUE	MAXIMUM CHANGE AFTER PITUITRIN TO:
Body weight	2.04 kgm.	1.88 kgm.
Serum osmotic pressure	0.908%	0.941%
Serum specific gravity	1.0246	1.0246
Red blood corpuscles	4.89 mil.	4.99 mil.
Hemoglobin	13.0 gm.-%	12.5 gm.-%
Hematocrit	33.0%	34.0%
Reticulocytes	5.4%	3.9%
Icteric index	4	5

A more striking result was obtained in those experiments designed to keep the blood picture constant following pituitrin injection. This was accomplished by a 48 hour dehydration of rabbits, previously on a wet diet. This procedure merely removed excess fluid from the gut and did not result in hemoconcentration. The animals were then placed on a diet of oats and injected with the usual dose of pituitrin. The fluid still remaining in the gut, plus small calculated amounts of water fed at intervals,

maintained a blood picture with respect to electrolyte concentration constant to within 5 per cent. As can be seen in table 5, this is reflected in a very uniform cell count.

The above results strengthen the viewpoint that the reduction in cell count following pituitrin is dependent upon a low serum osmotic pressure, and can be prevented by measures designed to maintain normal serum electrolyte concentrations.

Hemoglobin. Following pituitrin injection, the hemoglobin decreased along with the red blood cells. The amount of reduction varied with the type of anemia encountered. In the severe form due to actual cell destruction, as seen in table 2, the pigment declined to 4.5 grams per cent, or to one-third of its preinjection value. The red cell decrease at the height of the anemia, however, was considerably greater than that for hemoglobin, and thus the mean corpuscular hemoglobin rose from 22 to 33 micromicrograms or 50 per cent. This marked hyperchromia was also noted by Dodds and co-workers. It was undoubtedly due to the fact that the new cells rapidly produced by the bone marrow contained more hemoglobin. These young cells, as shown by reticulocyte counts, reached nearly 50 per cent at the low point of the anemia. The hyperchromia was due only to the increased cell volume because the mean corpuscular hemoglobin concentration remained constant.

When the anemia was entirely due to plasma dilution, the hemoglobin reduction paralleled that of the red corpuscles. This can adequately be accounted for purely on the basis of water retention as indicated by the serum osmotic pressure and specific gravity figures. In the experiment depicted in table 3, the hemoglobin decreased 16 per cent, the red cells 15 per cent, the serum electrolyte concentration 21 per cent; the mean corpuscular hemoglobin remained constant at 25 micromicrograms.

In those experiments in which dehydration preceded pituitrin injection and characterized by no evidence of cell destruction or anemia, the fall in hemoglobin from an elevated to a normal value was of the same order of magnitude as the decrease in red cells, and occurred with the return of the blood to a normal state of hydration. From the data in table 4, it can be seen that the hemoglobin decreased 17 per cent, the red cells 22 per cent, and the serum osmotic pressure 16 per cent.

Hematocrit. The hematocrit values following pituitrin injection varied with the type of anemia. In the severe form due to cell destruction, the volume of packed red cells dropped precipitously but not as markedly as the cell count. Consequently, the mean corpuscular volume increased. In the experiment shown in table 2, the red cells decreased 78 per cent, the hematocrit only 68 per cent; the mean cell volume rose from 55 to 80 cubic microns, a 45 per cent increase. Four days later, the m.c.v. reached 107, nearly a 100 per cent increase. This was due to the fact that the

new cells produced by the bone marrow in response to this anemia were large cells.

In the type of anemia produced primarily by dilution, the hematocrit fall paralleled the red cell decrease and can adequately be explained by the serum dilution which occurred. This can readily be seen in the experiment shown in table 3. The corpuscular volume remained unaltered.

In the dehydration experiments, the hematocrit value decreased slightly with the return of the blood to a state of normal hydration. Occasionally, as in the experiment shown in table 4, the hematocrit value fell less than the red cell count, and the mean cell volume thus *rose* somewhat. This was probably due to slight cellular swelling occurring as the serum osmotic pressure returned to normal after dehydration. In those experiments designed to keep the blood values constant, the hematocrit reading likewise remained unchanged.

Reticulocytes. Marked reticulocytosis reaching values of 50 per cent occurred in the severe form of pituitrin anemia. The reticulocyte response was so prompt that at the peak of the anemia it was already well developed and checked any further decline in red cells. The bone marrow responded rapidly and adequately to the need for cells. The return of reticulocyte percentages to preinjection values required over three weeks (table 2).

In those instances in which the anemia was primarily due to serum dilution, the percentage of reticulocytes either remained normal or rose moderately (4 to 10 per cent), varying directly with the degree to which cell destruction occurred.

In the dehydration experiments, as one would expect, no significant changes in the number of reticulocytes occurred subsequent to pituitrin administration.

Blood film. The cellular changes at the height of the severe anemia were very striking. As seen in the stained blood smear, the red cells showed marked variations in size, moderate poikilocytosis, and marked polychromatophilia. Many very large corpuscles having basophilic cytoplasm were present, and represent newly formed cells. Occasional normoblasts, erythroblasts and stippled cells were also seen. The differential white cell count revealed that the temporary leucocytosis which accompanied the hemolytic anemia was due to an increase in the neutrophils. Within four or five days after the peak of the anemia, the red cells were almost normal in appearance except that they were very large in size.

In the dehydration experiments the cells remained normal. In the dilution anemias, either no cellular alterations were evident or else there occurred a milder form of the same changes noted in the hemolytic variety. These changes were proportional to the degree to which the hemolytic factor entered in the dilution anemia.

Icteric index. In most experiments, the serum pigment was not detectably elevated despite the marked cell destruction. This is not surprising, however, for it is difficult to demonstrate increases in serum bilirubin in the rabbit. Occasionally, however, a definite elevation of icteric index occurred, but not above 12 to 15. In the dilution anemia, no increases were noted.

Urine urobilinogen was occasionally found but never in dilutions much higher than found in some normal animals.

In a few instances, free hemoglobin was demonstrated in the urine as indicated by positive chemical tests for blood, and negative examinations for red cells in the sediment.

Fragility. Interesting changes were observed in the "in vitro" fragility of the red blood corpuscles. As the osmotic pressure of the blood decreased the cells became more resistant to hemolysis in hypotonic saline. Conversely the cells of the dehydrated animals with abnormally high serum osmotic pressures were less resistant to hemolysis in the test tube. It seems apparent that the breaking point of a red cell is markedly affected by its previous environment, and the proportional change in osmotic pressure is perhaps more important than the absolute level of electrolyte concentration in determining the NaCl concentration at which the cell will rupture.

From the data presented it must be assumed that the red blood corpuscles are more fragile in vivo than in vitro. Cells did not rupture in the test tube until the concentration of NaCl was reduced to 0.50 per cent, whereas in the body cell destruction proceeded in a more favorable osmotic environment. This discrepancy might be attributed to the trauma attending circulation. It is also very likely that the reticulo-endothelial system removes injured cells whose membranes in the test tube are still impermeable to hemoglobin.

Discussion. One naturally views with reserve any claim for a new function of the posterior hypophysis. This is especially true when the newly proposed action is entirely different from any known physiological rôle of this gland. The only definite hormonal function of the posterior pituitary thus far demonstrated is that concerned with water metabolism (7), despite the numerous effects which may be obtained from posterior pituitary extracts.

The large doses of extract necessary to produce the characteristic blood changes demand explanation. Numerous papers could be cited describing the effects of pituitary preparations on various metabolic activities. Positive results usually depend upon the injection of huge amounts of extract. Dodds, in discussing the point, states "It seems at first sight that the quantity of material injected is so great as to make it doubtful whether the phenomena described are really exaggerations of a natural process. It must be borne in mind, however, that the B.P. pituitary extract is

standardized for the oxytocic and vasopressive principles in units which bear no relationship to the phenomena described." The fact remains, however, that Dodds' animals received 200 units of pitressin, a substance of marked antidiuretic activity. The extreme sensitivity of most species to the antidiuretic action of pituitary preparations is often overlooked. Theobald (8) has obtained antidiuresis in the dog and man with as little as 0.0005 unit of pitressin. Rabbits are still more sensitive, responding with diminished urine flow to 0.00002 unit (9). Thus the dose of pituitrin administered by Dodds and in the above experiments is 10,000,000 times the minimal quantity affecting the kidney. It is not surprising, therefore, despite the findings of Heller and Urban (10) on the inactivation of pituitrin in vivo, that this amount is sufficient to inhibit urine flow in the rabbit for over a week. In view of the effect of such prolonged antidiuresis on the blood chemistry of normally hydrated animals, surely it is not necessary to postulate an additional hormone to explain the accompanying cellular changes.

CONCLUSIONS

Pituitrin anemia in rabbits is the result of water retention causing blood dilution. The lowered osmotic pressure of the serum presents a distinctly abnormal environment for red corpuscles and causes their destruction. Measures designed to maintain the serum electrolyte concentration within normal limits after pituitrin will prevent this anemia.

REFERENCES

(1) Dodds, E. C. and R. L. Noble. Nature **135**: 788, 1935.
(2) Dodds, E. C., C. M. Hills, R. L. Noble and P. C. Williams. Lancet **1**: 1099, 1935.
(3) Hill, A. V. Proc. Roy. Soc. **127-A**: 9, 1930.
(4) Barbour, H. G. and W. F. Hamilton. J. Biol. Chem. **69**: 625, 1926.
(5) Darrow, D. C. and H. Yannet. J. Clin. Invest. **14**: 266, 1935.
(6) Gilman, A. This Journal **108**: 662, 1934.
(7) Gilman, A. and L. Goodman. Science **84**: 24, 1936.
(8) Theobald, G. W. Clinical Science **1**: 225, 1934.
(9) Levitt, C. J. Clin. Invest. **15**: 135, 1936.
(10) Heller, H. and F. F. Urban. J. Physiol. **85**: 502, 1935.

THE BLOOD VOLUME IN DIURESIS

A Study Employing the Colloidal Blue Dye T-1824 in Dogs Rendered Edematous by Plasmapheresis

WILLIAM A. EVANS, Jr. and JOHN G. GIBSON, 2nd

With the technical assistance of Leonard Landry

From the Medical Clinic, Peter Bent Brigham Hospital and the Department of Medicine, Harvard Medical School

Received for publication August 24, 1936

A colloidal blue dye, T-1824, was described by Dawson, Evans and Whipple (1) in 1920 in their search for dyes suitable for plasma volume determinations. Although many of its advantageous properties were recognized, it was not extensively used until Gregersen, Gibson and Stead (2) with the spectrophotometer again demonstrated its superiority over other dyes for plasma volume studies. The characteristics which recommend it are the constancy of the optical properties of solutions in serum or plasma, its very low toxicity (3), its slow rate of disappearance from the blood stream and finally its blue color which practically eliminates errors due to hemoglobin in the serum or plasma samples when dye concentration of samples is measured with the spectrophotometer. A detailed account of the effect of hemolysis on the estimation of dye concentration in serum samples and a method of correcting for it are described elsewhere (4).

To appreciate the validity of the plasma volume determinations by the dye method, it is necessary to have some knowledge of the fate of the intravenously injected dye after leaving the blood stream. The purposes of this investigation were to study changes occurring in the plasma volume during diuresis and to test the validity of the methods employed particularly in regard to the behavior of the dye in edematous dogs during the periods of formation and loss of the edema fluids.

Procedure. The diuretic experiments were carried out in the same manner as those described in previous work from this laboratory (5) with the exception that the control period was extended and the period of diuresis shortened so that the two were approximately equal. Diuresis was induced by the intravenous injection of salyrgan (0.05 cc./kgm.). As we have previously demonstrated, the magnitude of the diuresis is greatly increased in the presence of edema (6). As a further control on

the results, experiments were carried out on both edematous dogs, in which a very brisk diuresis was induced and normal dogs in which the diuresis was moderate.

Changes in the plasma and total blood volume during diuresis were studied in two ways. In one group of experiments two separate determinations were made, one before the onset of and the other at or shortly following the peak of the diuresis. In the other group changes were observed during the diuresis in the concentration of the dye in the blood stream after it had reached a slow and constant rate of disappearance. The first method has the advantage of giving direct evidence of an absolute change in blood volume, while by the second relative changes in volume can be observed at frequent intervals during various phases of the diuresis.

The method of single and repeated volume determinations has been described elsewhere (3). In these experiments 4 cc. of 0.3 per cent T-1824[1] in saline was the amount usually employed for each determination. The disappearance slope over a period of 30 minutes after dye injection was determined by taking six serum samples. The sample of serum or plasma taken immediately before the injection of the dye together with the subsequent samples were compared directly in two cells of 10 mm. depth in the spectrophotometer. The readings so obtained give the increment of optical density (ΔD) due to the injected dye alone regardless of any dye already present at the time of the determination (3). The disappearance slope was extrapolated to the time of injection and the value of dye concentration at this point used in the calculation of the plasma volume. Three or four hematocrit determinations were carried out by the method previously described (4) during the 30 minute period and an average of the values taken for the computation of the total blood volume. All blood samples were obtained from a jugular vein with an interval of at least one minute after the needle had entered the vein and the stasis had been relieved before the blood was withdrawn. For each volume determination a total of 30 to 40 cc. of blood was withdrawn.

The rate of disappearance of dye from the blood stream following the injection of large amounts is at first relatively rapid; this rate diminishes progressively as the dye concentration in the blood falls, and after a time a constant very slow rate of elimination obtains (4) (7) (8). In the second group of experiments a large quantity of dye was injected 15 to 18 hours before the experiment. During the control period of the experiment, the dye concentration and rate of elimination were obtained from samples of serum withdrawn at intervals of 30 to 40 minutes for a period of $2\frac{1}{2}$ to 3 hours. Salyrgan was then given; during the ensuing period

[1] All T-1824 used in these experiments was from the same lot, as supplied by the Eastman Kodak Company.

of diuresis samples were obtained at intervals corresponding to those taken during the control period and deviation of the dye concentrations of these samples from the disappearance slope observed in the control period was interpreted as a change in the blood volume. An increase in the concentration of the dye was taken to indicate a reduction in plasma volume, and vice versa. Blood for hematocrit readings and plasma samples was also obtained. The total quantity of blood withdrawn in such an experiment was 70 to 80 cc.

To render the animals edematous plasmapheresis was carried out twice daily, and the animals were given a low protein diet and liberally supplied with 0.85 per cent sodium chloride solution by mouth. It was found that by withdrawing 650 to 700 cc. of blood at each bleeding the edema would become apparent in from three to five days. On the day that the edema first became noticeable, the blue dye T-1824 was injected intravenously in amounts of 15, 18, 30 or 50 mgm. daily, the amounts varying in different experiments. These are quantities giving plasma concentrations far in excess of those encountered in single plasma volume determinations. (Ten to 15 mgm. provide the optimal dye concentration in 20 mm. cells for plasma volume determinations in the adult human.) All food and fluids were withheld and no dye given or plasmapheresis carried out for at least fifteen hours before edema fluids were withdrawn for analysis or the diuretic experiments begun. The most readily available edema fluid was ascitic fluid, and this was withdrawn by simple paracentesis. Edema fluid from the subcutaneous tissues was obtained by puncturing the skin near the paw of one of the hind legs and gently milking the leg. Occasionally, such fluid was blood-tinged, but with patience it was possible to obtain samples containing only a very few red cells and presumably uncontaminated by blood. Pleural fluid was obtained twice by thoracentesis. All fluids so obtained were centrifuged before analysis and chemical examination.

Results. *Edema fluids.* Samples of the ascitic, pleural and subcutaneous tissue fluids were examined in test tubes against a white background and compared with high dilutions of dye. The color intensity of T-1824 is such that a distinct blue tinge is imparted to aqueous solutions in dilutions as high as one part in 2,000,000. In none of the samples (with the one exception described below) could any trace of a blue color be detected. By this method it was apparent that dye was present, if at all, in dilutions greater than 1/2,000,000. During the time that the edema fluid was forming the dye concentration of the plasma was maintained for several days at dilutions of from 1/30,000 to 1/150,000 by repeated injections. The optimal concentration of dye for estimation of the plasma volume with the spectrophotometer in cells of 20 mm. depth is about 1/150,000. Thus, if no dye can be detected in such edema

fluids when the blood concentration is maintained at much higher levels for several days, it can be fairly concluded that no appreciable quantity of dye accumulates in the edema fluids during a simple blood volume de-

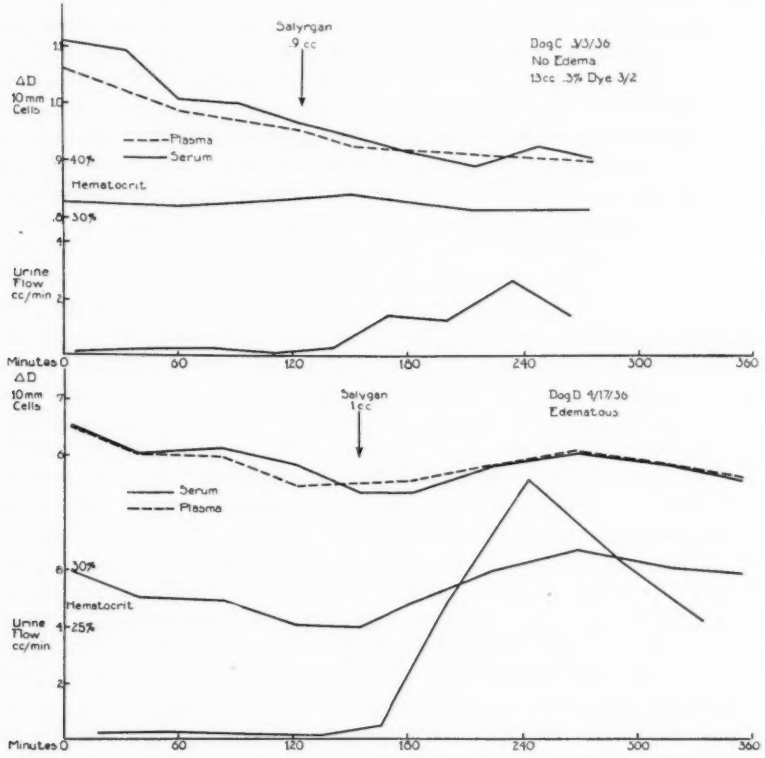


Fig. 1. Dog C. Observations of dye concentration 17 hours after the injection of a large amount. The animal is not edematous, the diuresis is moderate and there are no appreciable changes in the slope of the dye concentration or hematocrit values during the diuresis.

Dog D. Observations of dye concentration after the injection of a large amount in an edematous animal exhibiting a brisk diuresis. Concurrent with the diuresis there is a rise in the dye concentration and hematocrit values indicating a diminishing blood volume.

termination. It is to be noted that such edema fluids have a very low protein content, being of the order of magnitude of 0.1 gram per cent in the samples which were analyzed for us.

In one experiment a single massive injection of dye was made in an

edematous animal which had been given frequent injections previously, and samples of urine, ascitic and edema fluids were withdrawn two hours later. While 25 mgm. was sufficient to give the serum a deep blue color, in this instance 250 mgm. was given. In this dosage (a total of 400 mgm. in 21 days or 20 mgm. per kgm.) toxic effects may be expected (3), and the results are hardly physiological, but even here no dye could be detected in the edema fluid or urine, and only the faintest amount in the ascitic fluid. At the time these fluids were withdrawn there was definite staining of the skin and mucous membranes, and dye could be seen in the purulent material from a small area of superficial skin infection. This animal died ten days later, and on examination the organs showed a deep

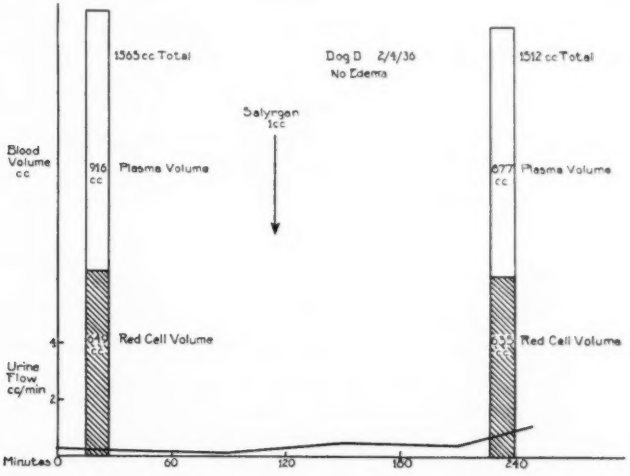


Fig. 2. Direct estimation of plasma and blood volume before and during diuresis. The animal is not edematous, the diuresis is minimal, and the changes are slight.

blue staining of the skin, tendinous structures, heart valves and aorta, as well as of the serous and mucous membranes of the gastro-intestinal tract. The lungs and muscular tissues contained no dye to gross examination, while the kidneys, lymph nodes, liver and spleen were very intensely stained. The cause of death was not otherwise apparent.

The attempts to estimate the changes in plasma volume during diuresis confirmed in every way the conclusions previously drawn from a study of the changes in protein concentration (5). No changes were detectable prior to the onset of the diuresis, and concurrent with the diuresis there was a diminution in the volume of plasma and an increase in the concentration of its non-diffusible contents in a measure proportional to the mag-

nitude of the diuresis (figs. 1–3). However, certain obstacles appeared in these experiments with dogs which made it seem impossible to quantitate the effect in an exact manner. The most important of these was the effect of repeated blood sampling alone on certain of the blood constituents. This effect gave an apparent dilution of the red blood cells and the dye out of all proportion to the small amount of blood withdrawn. It was sufficiently constant and of such a degree as to be inexplicable by the manner in which the samples were taken or the behavior of the dog during the period of sampling. In the experiments reported by Bryan et al. (5) a fall in the protein concentration from sampling alone frequently

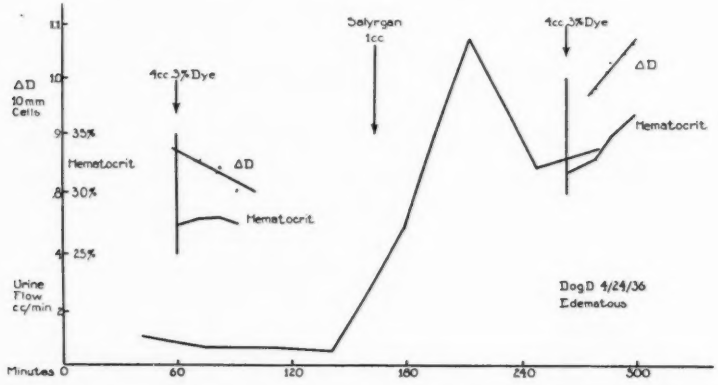


Fig. 3. An attempt to estimate the plasma and blood volumes in an edematous animal before and during a brisk diuresis. The same quantities of dye were used in the two determinations. The higher concentrations of dye in the determination during the diuresis indicates a smaller plasma volume. The distortion of disappearance slope, apparently as a result of the diuresis, precludes the estimation of the plasma volume in the usual manner. Comparable changes are seen in the hematocrit values.

was detectable. Similar findings in dogs are reported by Hemingway, Scott and Wright (8) who described a decrease in hemoglobin and dye concentrations in samples obtained at hourly intervals and quoted the similar observations of Chanutin, Smith and Mendel (9) on the hemoglobin concentrations of blood samples obtained in a comparable manner.

The observations here pertain to red cell (hematocrit) and dye concentrations observed in samples.

In making single volume determinations, blood samples for hematocrits were obtained at approximately 10 minute intervals over a period of 30 minutes. In 9 out of 10 experiments there was a fall in the hematocrit

value of successive samples, the average value of successive samples being as in table 1. From the data obtained in single volume determinations no conclusions can be drawn as to the effect of repeated sampling on dye concentration and hence upon the disappearance slope. It seems probable, however, that the dilution effect does increase the apparent disappearance slope and tends to influence the calculated volume. This error is minimized by the method of extrapolation previously described (4).

However, the effect was demonstrable in a striking manner in the efforts to obtain a disappearance slope of the dye 15 to 17 hours after the injection of a large amount. The fall in dye concentration observed at this time in samples taken at 30 to 40 minute intervals in a $2\frac{1}{2}$ to 3 hour period gave apparent disappearance rates from 5 to 11 per cent an hour. These values cannot represent true disappearance rates for, if such were the case, the dye would already have largely or entirely disappeared. In reality the

TABLE 1

Average values of hematocrits in blood samples taken at 10 minute and at 30 to 40 minute intervals based on a value of 100 for the first sample

SAMPLE	PER CENT VALUE	
	10 minute interval	30-40 minute interval
I	100	100
II	97.8	97.3
III	96.3	94.2
IV	95.0	88.7
V		89.6

true disappearance rate is minimal at the time of these observations. A fall in the hematocrit values comparable to that in the 10 minute samples was observed in 5 of 6 experiments. The average values of successive samples are given in table 1.

We have recently made similar observations on human subjects, and the effect here is also definite and constant though to a less marked degree.

A further difficulty was encountered in the single determination of the plasma volume while rapid changes were taking place. Figure 3 illustrates an attempt to determine the plasma volume shortly after the peak of a very vigorous diuresis. The observations over a half-hour period indicate that the dye instead of disappearing in the usual way actually increased in concentration, and there was a corresponding rise in the hematocrit values. While this increasing concentration of the blood during the period of diuresis illustrates the effect of the diuresis, it vitiates the absolute determination of the plasma volume during such changes.

Discussion. Our findings regarding the fate of intravenously injected T-1824 indicate that it leaves the blood stream slowly, that it is not detectable in and does not accumulate in edema fluids, and that in large doses the tissues of many organs become stained. From the failure to find the dye in the edema fluids, one may conclude either that the dye does not pass the endothelium of tissues where the edema fluid is forming, or that if it does pass the endothelium it is promptly removed from the intercellular spaces. It is known that the endothelium is permeable to a varying degree in different organs, and it is quite possible that in low concentrations the dye is removed exclusively in such organs as the liver, spleen and kidney, while in higher concentrations the dye passes the endothelium more generally. The widespread staining of the tissues after large doses and the failure to find any dye in the edema fluids suggests that the dye is promptly fixed by the tissues as it leaves the blood stream. This is in accord with numerous observations concerning the great rapidity with which vital dyes are removed after injection into the peritoneal and other serous cavities. After the dye becomes fixed in the tissues, it disappears slowly over a long period and probably re-enters the blood stream in small amounts (7). However, this process is so protracted that the dye re-entering the circulation could not conceivably interfere with observations of serum dye concentration made over a period of a few hours. While these observations pertain to edematous dogs it seems proper to assume that the endothelium of these animals is normal and that the conclusions are applicable to normal dogs also. On the other hand where the permeability of the endothelium is altered as in areas of acute inflammation or in kidneys showing albuminuria we have observed the dye in high concentration as in the serous exudate of patients with burns and in the albuminous urine of nephritic patients. This may be linked with the apparent affinity of the dye for protein for, while such fluids are rich in protein, the dye is not to be found in such protein poor fluids as normal urine and edema fluids associated with hypoproteinemia.

The validity of the conclusions drawn from studies of the protein concentration during diuresis depended upon the assumption that no protein entered the blood stream during the observation period (5). Nonnenbruch (10) believed such an assumption was not warranted and indeed the possibility of a protein shift could not be denied. In the present experiments with repeated single blood volume determinations and observations of dye concentration during the period of diuresis, the possibility of reentrance of appreciable quantities of the dye from tissues and edema fluid would appear to be excluded. The results indicate that with the onset of the diuresis there is a diminution in the plasma volume proportional to the magnitude of the diuresis.

CONCLUSIONS

1. The colloidal blue dye, T-1824, when injected intravenously into a dog being rendered edematous by plasmapheresis in amounts sufficient to cause vital staining does not accumulate in the edema fluids.

2. The presence of this edema does not invalidate the methods employed for the determination of the plasma volume.

3. Observations of the dye concentration in the blood serum during diuresis induced by salyrgan give evidence by direct and indirect methods of a diminution of the plasma volume during the diuresis.

4. A dilution effect of certain of the non-diffusible blood constituents observed in successive blood samples is described. This phenomenon is an obstacle to the exact quantitative study of dye disappearance and changes in blood concentration.

REFERENCES

(1) Dawson, A. B., H. M. Evans and G. H. Whipple. This Journal **51:** 232, 1920.
(2) Gregersen, M. I., J. G. Gibson and E. A. Stead. This Journal **113:** 54, 1935.
(3) Gibson, J. G. 2nd and M. I. Gregersen. This Journal **113:** 50, 1935.
(4) Gibson, J. G. 2nd and W. A. Evans. To be published.
(5) Bryan, H. A., W. A. Evans, M. N. Fulton and E. A. Stead. Arch. Int. Med. **55:** 735, 1935.
(6) Evans, W. A. The effect of changes in salt and water metabolism upon salyrgan diuresis. Medical Papers Dedicated to Dr. Henry A. Christian, 1936.
(7) Smith, H. P. J. Exper. Med. **51:** 379, 1930.
(8) Hemingway, A., F. H. Scott and H. N. Wright. This Journal **112:** 56, 1935.
(9) Chanutin, A., A. H. Smith and L. B. Mendel. This Journal **68:** 444, 1924.
(10) Nonnenbruch, W. Ergebn. d. inn. Med. u. Kinderh. **26:** 119, 1924.

THE COAGULATION DEFECT IN SWEET CLOVER DISEASE AND IN THE HEMORRHAGIC CHICK DISEASE OF DIETARY ORIGIN[1]

A Consideration of the Source of Prothrombin

ARMAND J. QUICK

From the Department of Pharmacology, Marquette University School of Medicine, Milwaukee

Received for publication August 24, 1936

The importance of prothrombin deficiency as a primary cause of hemorrhage has been demonstrated by the studies of Quick, Stanley-Brown and Bancroft (1), who found a marked reduction of this clotting factor in various types of jaundice. The desirability of finding for experimental purposes other hemorrhagic conditions in which a decrease of prothrombin occurs, led to the study of the curious and relatively little known sweet clover disease. Schofield (2) was the first to establish the fact that feeding improperly cured sweet clover hay produced in cattle a serious alteration in the coagulability of the blood which often resulted in fatal hemorrhage. Much of the present information concerning the disease is due to the splendid and thorough investigations of Roderick (3, 4). He showed that the clotting defect in sweet clover disease is due to a drastic reduction of the prothrombin in the blood. This finding, which the writer (5) has recently confirmed, suggested the possibility that sweet clover disease might be employed as a means for securing new and valuable information concerning the source and production of prothrombin, and with this aim the present investigation was undertaken. In the course of the work, it became apparent that the coagulation defect of sweet clover disease might be related to the hemorrhagic condition that can be produced in chicks by dietary means as recently reported by Dam and Schønheyder (6, 7), Schønheyder (8), Almquist and Stockstad (9), and Almquist (10). By putting chicks on the diet of Almquist, the disease was readily developed, and a study of the blood disclosed a pronounced fall of prothrombin, a finding which has also been recently reported by Dam, Schønheyder, and Tage-Hansen (11). In the present paper a quantitative study of the

[1] The writer takes this opportunity to express his thanks to Dr. William K. Smith of the University of Wisconsin, who kindly furnished the toxic sweet clover hay employed in this study, and who gave valuable suggestions.

prothrombin in both diseases is presented, and an attempt is made to correlate the causes of the reduction of prothrombin especially since such an effort might lead to the development of a new approach to the study of the hemorrhagic diathesis of obstructive jaundice.

EXPERIMENTAL. *Production of sweet clover disease.* Adult rabbits were fed toxic sweet clover hay *ad libitum*, and in addition were given 15 grams of oats a day. Blood was usually taken daily. One and eight-tenths cubic centimeters were drawn directly into a syringe containing 0.2 cc. of 0.1 M sodium oxalate. The frequent removal of this small amount of blood did not in itself produce any demonstrable degree of anemia.

Hemorrhagic chick disease (vitamin K deficiency). White Leghorn chicks, 8 days old, were put on the diet of Almquist (10), which consists of 17.5 parts of fish meal extracted with ether, 7.5 parts of brewers' yeast extracted with ether, 73 parts of ground polished rice, 1 part of sodium chloride containing small amounts of ferrous and cupric sulfates, and 1 part of cod liver oil. Part of the chicks used as normal controls were kept on a start mash, the others on the basal diet of Almquist plus 2 per cent ground alfalfa. Blood was drawn from the brachial vein with a hypodermic needle into a syringe containing 0.1 cc. 0.1 M sodium oxalate. Since 1 cc. of blood was taken, which is a comparatively large amount, no chick was bled oftener than once in 5 days.

Quantitative determination of prothrombin. In a former publication, the writer (12) showed that when oxalated plasma is mixed with an excess of thromboplastin and then is recalcified with an optimum amount of calcium, a minimum and constant clotting time is obtained. By increasing dilution of the plasma either with normal physiological saline or with prothrombin-free plasma (prepared according to the author's aluminum hydroxide method (12)), the coagulation time is progressively increased, thus showing that the speed of clotting is dependent upon the concentration of prothrombin. No other factor, except a change in the electrolyte concentration, which can readily be controlled, was found to influence the coagulation time. Therefore it was possible to develop a method for determining quantitatively plasma prothrombin in terms of per cent of the normal. By means of diluting normal rabbit plasma either with normal saline or prothrombin-free plasma, the clotting times corresponding to various concentrations of prothrombin were obtained and recorded in figure 1. The exact details of the method were as follows: The original plasma was obtained by drawing 9 cc. of blood into a syringe containing 1 cc. of 0.1 M oxalate, rapidly mixing and centrifuging. In a small test tube 0.1 cc. of the clear plasma was mixed with 0.1 cc. of thromboplastin emulsion (prepared from rabbit brain (12)), and quickly recalcified with 0.1 cc. of 0.025 M calcium chloride. The tube was placed in a water bath maintained at 38–40°C., and the exact time required for a solid clot

determined. Progressive dilutions of the original plasma were made, and the coagulation time determined in the same manner. The curve obtained in figure 1 is a composite of numerous determinations. Only thromboplastin which had a high degree of activity, i.e., caused recalcified plasma to clot in 7 seconds, was employed.

It should be noted that the coagulation time is relatively little delayed until the prothrombin concentration drops below 20 per cent of normal.

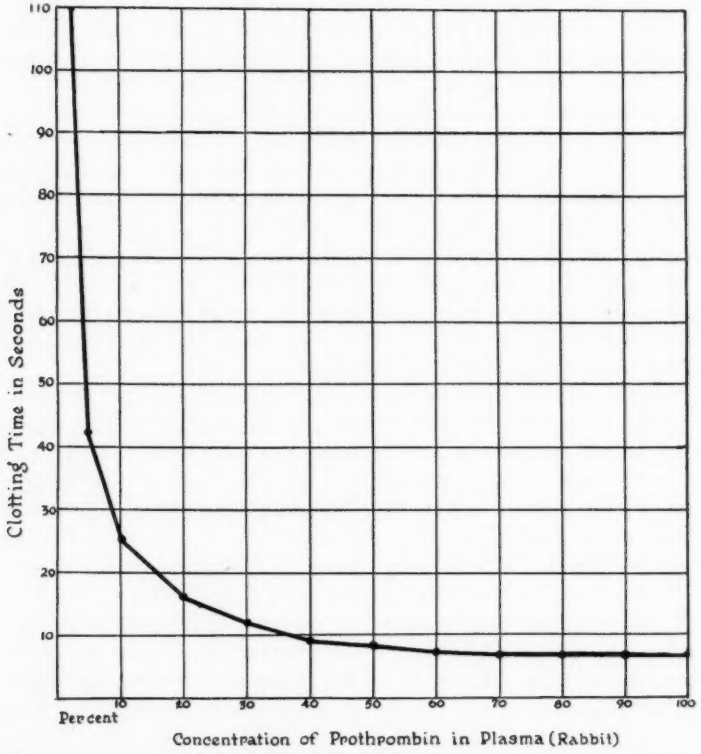


Fig. 1. The relation of the clotting time (of recalcified plasma with excess thromboplastin) to the concentration of prothrombin in rabbit plasma.

The range approximately between 10 or 20 and 100 per cent can be considered the margin of safety and represents roughly an amount in excess of that required for normal coagulation. The great excess of prothrombin has also been noted by Warner, Brinkhous, and Smith (13). It can readily be seen from the curve that the determination of prothrombin below 2.5 per cent becomes unsatisfactory since the clotting time rapidly increases as the prothrombin diminishes.

For the estimation of prothrombin in chicken plasma, the same technique was employed. Thromboplastin was obtained from chicken brain freed as completely as possible from all traces of blood. A 10 per cent saline emulsion of fresh or carefully dehydrated brain was employed. Difficulty was often encountered in obtaining a dehydrated product having a uniform and maximum activity. No attempt was therefore made to construct a curve of prothrombin values as in the case of rabbit blood; and the values given for prothrombin in chicken blood should be considered only roughly quantitative. Nevertheless, when the clotting time, which is normally 10–11 seconds according to the writer's method, is prolonged to 45–50 seconds, which corresponds to a prothrombin concentration of less than 10 per cent of normal, a distinct hemorrhagic tendency manifests itself.

RESULTS. *The effect of feeding toxic sweet clover.* Figure 2 and table 1 graphically show the action of toxic sweet clover. In a few days, occasionally even on the third day following the feeding of spoiled sweet clover hay, a precipitous drop of the plasma prothrombin occurs. After the initial fall, the decrease is less rapid, but nevertheless is progressive. When the concentration of prothrombin sinks below 10 per cent, the hemorrhagic tendency becomes very apparent. In the earlier work, when the blood was obtained by heart puncture, the animal at this stage invariably died from a hemopericardium, for apparently the delayed clotting prevented the formation of a clot to plug the tiny perforation in the cardiac wall. With the extreme low concentration of prothrombin, difficulty is encountered in stopping bleeding even in such trivial small wounds as are produced by needle pricks. Transfusion with citrated blood dramatically improves the condition and the analysis of the blood shows a pronounced restoration of the prothrombin concentration in the plasma. But unfortunately the effect is very temporary since the prothrombin is back to the previous low level the following day. Significantly, defibrinated blood which has been allowed to stand an hour had scarcely any influence in elevating the prothrombin of the plasma. Curiously, Roderick found that defibrinated blood controlled fairly effectively acute hemorrhage in sweet clover disease. It is entirely probable that his good results can be attributed to the thrombin remaining in the defibrinated blood and to the restoration of blood volume. The fact that no increase in prothrombin occurs after the injection of serum which has been sufficiently aged to insure a fairly complete conversion of prothrombin to thrombin, strongly refutes the theory of Mills (14) that prothrombin can and is regenerated from thrombin.

Diet is the important means for controlling the disease. The incorporation of 5 per cent of dehydrated alfalfa meal with the toxic hay was found sufficient to prevent the development of the disease or even any

demonstrable reduction of prothrombin. Strangely, however, the addition of the same amount of alfalfa to spoiled sweet clover after the prothrombin had been markedly reduced did not restore the latter to normal (table 2). Nevertheless, the alfalfa did arrest the disease and maintained the prothrombin concentration at a constant level. Significantly, alfalfa which was thoroughly extracted with ether appeared in all respects as active as the unextracted material. Merely stopping the feeding of toxic

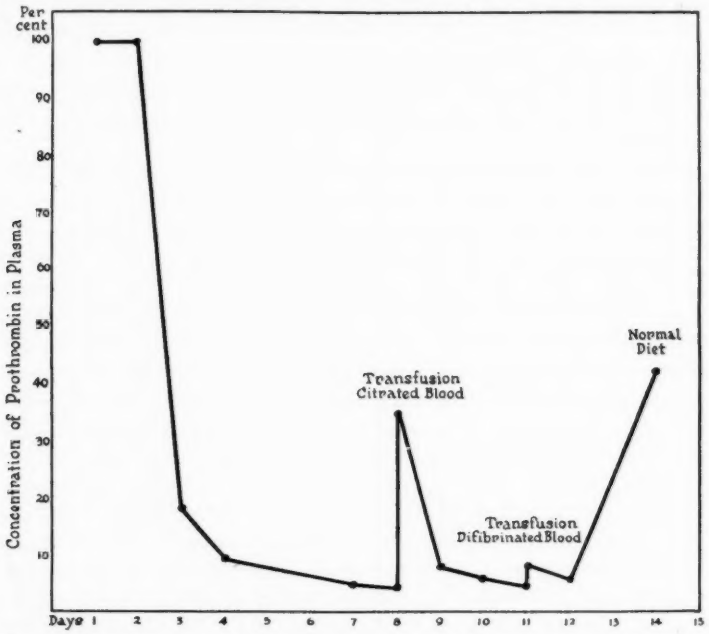


Fig. 2. The reduction of plasma prothrombin following the feeding of spoiled sweet clover hay. In each of the two transfusions, 30 cc. of blood were given. The defibrinated blood was allowed to age one hour. A specimen of blood for the determination of plasma prothrombin was taken 3 hours after the transfusion. The toxic hay was replaced by a normal diet on the 12th day.

hay does not cure the disease, in fact, the prothrombin continues to drop, but alfalfa promptly brings about a pronounced replacement of prothrombin. Inspection of Roderick's experiments clearly shows that alfalfa was effective in counteracting the toxic action of sweet clover, but apparently Roderick was not greatly impressed and seems to adhere to the opinion that feeds are protective merely in proportion to the extent to which they replace spoiled sweet clover.

Hemorrhage in sweet clover disease can be definitely attributed to lack

of prothrombin and from the observations made in the present study, it can be stated that the severity of the hemorrhagic condition runs parallel with the degree of prothrombin depletion. The importance of this clotting factor in sweet clover disease was first demonstrated by Roderick in a qualitative way, for he succeeded in restoring the coagulability of the blood in the advanced stage of the disease by adding prothrombin prepared by Howell's method from normal blood. No other coagulation factor appears to be altered. Roderick has shown that fibrinogen, calcium, platelets, and inhibitory substances all remain within normal limits. Similar observations have been noted by the writer. No heparin-like anti-

TABLE 1

The effect of spoiled sweet clover hay on the prothrombin of the plasma

Male rabbit, weight 2.9 kgm.

DATE	CLOTTING TIME*	PROTHROMBIN CONCENTRATION IN PLASMA	REMARKS
	seconds	*per cent*	
July 6	7	100	Feeding of toxic sweet clover hay started
July 8	15	22	
July 9	21	15	
July 10	30	8	
July 13	80	2.5	
July 14	320	>2.5	Marked hemorrhagic tendency
	38	5.5	3 hours after a 30 cc. transfusion with defibrinated blood
July 15	<600†	>2	Emulsion of an ether extract of alfalfa meal given orally. Feeding of toxic hay stopped
July 16	No clot		Ether extract of alfalfa given
July 17	No clot		Profuse bleeding from mouth. Animal sacrificed

* By the writer's method for determining prothrombin.

† Coagulation time of the blood by the Lee-White method was 2 hours.

thrombin could be detected by the method recently developed by the author (15).

Hemorrhagic chick disease. When chicks were placed on Almquist's diet, which contains all the known vitamins, the prothrombin gradually dropped (table 3). Coincidentally with the low prothrombin, a distinct tendency to hemorrhage developed. In the advanced stage oozing from a pin feather would sometimes continue for 24 to 36 hours, and often it was difficult to stop bleeding after a venous puncture with a hypodermic needle. The chicks which were kept on a complete diet or on the basal mixture of Almquist containing alfalfa showed no alteration in prothrombin.

By incorporating 1 per cent of dehydrated alfalfa meal in the basal diet, a chick in which the prothrombin had been reduced to a seriously low level could readily be saved. This amount of alfalfa however does not appear to be adequate for the complete restoration of the prothrombin to normal, but is sufficient to bring it well into the range which can be designated as the margin of safety. When the alfalfa was increased to 2 per cent, the

TABLE 2

The influence of fasting and of alfalfa on the toxic action of spoiled sweet clover

DATE	CLOTTING TIME	PROTHROMBIN CONCENTRATION IN PLASMA	REMARKS
		Male rabbit, weight 2 kgm.	
	seconds	*per cent*	
Jan. 28–30	7	100	Spoiled sweet clover hay fed
Jan. 31	15	22	
Feb. 1–2			Animal fasted
Feb. 3	38	5.5	Animal died from a hemopericardium following a heart puncture
		Male rabbit, weight 2.4 kgm.	
July 31	7	100	Spoiled sweet clover hay fed
Aug. 3	10	40	
Aug. 4	17	19	
Aug. 5	37	7	5 per cent extracted alfalfa meal fed with the toxic hay
Aug. 6	24	11	Feeding the same as on the previous day
Aug. 7	28	9	Feeding the same as on the previous day
Aug. 8	26	9.5	Feeding the same as on the previous day
Aug. 9			5 per cent of unextracted alfalfa meal fed with the toxic hay
Aug. 10	28	9	Feeding the same as on the previous day
Aug. 11	30	8.5	10 per cent extracted alfalfa meal fed with the toxic hay
Aug. 12	13	27	Feeding of toxic hay stopped; 5 grams of extracted alfalfa meal fed

The prothrombin concentration remained normal in all experiments in which 5 per cent of alfalfa meal, whether extracted or unextracted, was fed immediately with the toxic hay.

prothrombin rose to the normal level. Alfalfa extracted with ether lost its power to bring about prothrombin production and even with 2 per cent incorporated in the basal diet, the concentration of prothrombin continued to drop. These observations are fully in accord with the results reported by Almquist and Stokstad.

Curiously, the chicks which were on the basal diet failed to have a nor-

mal growth of feathers. Large areas especially over the back remained bare, and stubble quills became filled with non-coagulable blood. Soon after alfalfa was incorporated in the diet, a striking growth of feathers occurred. Whether this is a constant finding is doubtful since neither Dam and Schønheyder, nor Almquist mentions such an observation.

TABLE 3

Changes in the prothrombin concentration of the blood induced by vitamin K deficiency

DATE	CLOTTING TIME WITH APPROXIMATE CONCENTRATION OF PROTHROMBIN IN PARENTHESES*	REMARKS
July 6	11 (100), 11 (100), 10 (100)	10 chicks put on Almquist's diet; 5 chicks used as controls†
July 7	11 (100)	
July 10	17 (40), 13 (60)	
July 13	14 (55), 17 (40)	
July 14	24 (22)	
July 15	24 (22), 25 (20)	
July 17	28 (19), 19 (35)	
July 20	23 (25), 25 (20)	
July 21	30 (18), 23 (25)	
July 24	46 (10), 32 (17), 32 (17)	Chicks divided into groups 1 and 2
	Group 1	
July 27	50 (10), 37 (14)	Almquist's diet + 2 per cent ether extracted alfalfa meal. Diet started July 25
July 29	50 (10), 40 (12)	Distinct hemorrhagic tendency
	Group 2	
July 27	19 (35), 22 (27), 17 (40)	Almquist's diet + 1 per cent alfalfa meal. Diet started July 25
July 28	25 (20), 16 (45)	
July 29	18 (37), 19 (35)	
Aug. 3	18 (37), 20 (32)	Almquist's diet + 2 per cent alfalfa meal
Aug. 5	11 (100), 10 (100), 11 (100)	

* By progressively diluting chicken plasma with physiological saline and determining the clotting time after adding excess thromboplastin and recalcifying, a series of values was obtained which was used for the determination of prothrombin in the plasmas above.

† No alteration in the prothrombin of the blood was found in any of the control chicks.

COMMENT. The results obtained from the study of sweet clover disease as well as of the hemorrhagic chick disease furnish important new information concerning prothrombin, but the interpretation and correlation of the present findings is a difficult problem. The work of Dam and

Schønheyder and of Almquist and Stokstad clearly leads to the conclusion that hemorrhagic chick disease is due to a vitamin deficiency. Dam (16) has proposed the name vitamin K, and Almquist (10) has recently succeeded in obtaining a highly concentrated preparation. The new vitamin is fat-soluble and is found in the non-saponifiable fraction of an ether or petroleum ether extract. Alfalfa constitutes a rich source for making a highly potent preparation, but many other foods contain the vitamin. Hog's liver fat contains a relatively large amount and it is possible that the liver may be a storage depot. The studies on vitamin K point to the important conclusion that the substance is essential for the production of prothrombin in the chick. The exact function of this vitamin in the synthesis of prothrombin is puzzling since there is no conclusive evidence that prothrombin contains a lipoid group. Whether the vitamin constitutes a component necessary in the building of the prothrombin molecule remains to be demonstrated, but it seems fairly certain that the chick cannot produce prothrombin if it is deprived of this newly discovered food element. Failure thus far to produce the disease in other animals does not rule out the possibility that it may occur and that other species likewise require an anti-hemorrhagic vitamin. It is more than probable that the condition may be encountered clinically. As already mentioned, the writer with Stanley-Brown and Bancroft found a marked drop in the prothrombin concentration of the blood in a certain number of patients having obstructive jaundice. It should be noted that peculiarly suitable conditions for the development of a vitamin deficiency are found in these patients: their long restricted diet is very apt to lead to an inadequate intake of vitamins, and the absence of bile in the intestines to a poor absorption especially of the fat soluble type. The fact that the blood of these patients has a normal fibrinogen content indicates that the fall in prothrombin is probably not due primarily to liver injury. In acute hepatitis, such as is produced by chloroform or phosphorus, a pronounced drop of both prothrombin and fibrinogen occurs, as Warner, Brinkhous, and Smith (13) have shown, and the writer has confirmed (unpublished studies), using the quantitative method for prothrombin described in this paper. It has been observed that certain patients having a low prothrombin rapidly regain the normal level when their condition permits a more adequate diet, a finding which speaks against an organic injury of the liver. The prothrombin deficiency which Hawkins and Brinkhous (17) recently reported in bile fistula dogs also might well be explained on the basis of an inadequate absorption of a substance similar or identical with vitamin K due to the absence of bile in the intestines of these dogs.

The drastic reduction of prothrombin in sweet clover disease does not appear to be directly related to a deficiency of vitamin K. Not only does

the crude ether extract of dehydrated alfalfa, which is rich in this vitamin, fail to show any curative effect, but also alfalfa which has been thoroughly extracted with ether seems fully to retain its effectiveness in counteracting the toxic action of sweet clover. While the evidence points against any direct relation of vitamin K to sweet clover disease, the possibility that the latter may nevertheless to a certain extent be a deficiency disease warrants consideration. No explanation for the action of spoiled sweet clover has been offered, but it seems logical to conclude that the toxic hay contains a substance which either directly destroys or inactivates prothrombin, or has an inhibitory action on the mechanism responsible for the production of this clotting factor. The prompt recovery observed when the toxic hay is replaced by normal feed fairly definitely precludes an organic injury in the organ or tissue concerned with the production of prothrombin. One cannot arrest the progressive drop of prothrombin merely by stopping the feeding of spoiled hay, but strikingly, even a small quantity of alfalfa will bring about a prompt and pronounced rise in plasma prothrombin. These findings suggest that toxic sweet clover brings about a profound depletion of prothrombin, and that to replace this loss, the organism requires a material of exogenous origin, which apparently is abundant in alfalfa. The animal appears to be able to store this accessory factor, for it is very difficult to produce sweet clover disease in animals that have been fed relatively large amounts of alfalfa. This explains why some animals are far more resistant than others to the same lot of spoiled hay. The large reserve undoubtedly accounts for the infrequency of a hemorrhagic diathesis even after a prolonged period of faulty diet. There is of course the possibility that alfalfa contains a substance which directly neutralizes the toxic principle of the hay, but it seems more reasonable to assume that alfalfa supplies an accessory factor required by the organism to replete the store of prothrombin, which the toxic sweet clover has reduced to an extremely low concentration. This seems particularly plausible since the chick definitely requires an accessory food factor for synthesizing prothrombin. Since alfalfa seems to supply both vitamin K and the hypothetical substance that counteracts the toxic action of sweet clover, it is probable that a relationship between these two substances exists, although the fact that one is soluble in ether whereas the other is not, proves that they are not identical. Speculation as to the rôle played by these unknown food constituents in the synthesis of prothrombin is futile at our present stage of information. It must be remembered that experimetal evidence indicates that the prothrombins in different animals are not identical as the author has previously shown (12). Thromboplastin prepared from chicken brain or lung acts specifically on chicken plasma, clotting it on recalcification in about 11 seconds. This same preparation has scarcely any accelerating action on rabbit plasma,

for under the same conditions of recalcification coagulation occurs only in 45 to 60 seconds. Furthermore, rabbit thromboplastin which brings about coagulation of its own plasma in 7 seconds is almost inert when mixed with chicken plasma. One's curiosity is aroused on how the prothrombin molecule is constructed. Perhaps these accessory factors are compounds or contain structural entities required in the synthesis of prothrombin. The observation of Dam and Schønheyder (7) that aluminum oxide adsorbs vitamin K so firmly that it cannot be eluted is especially interesting since the writer has demonstrated that aluminum hydroxide adsorbs almost quantitatively prothrombin from plasma and that in this case too the union is so firm that the prothrombin cannot be recovered.

A certain number of important and practical conclusions can be drawn from the results of the present investigation. Both sweet clover disease and vitamin K deficiency emphasize the importance of prothrombin depletion as a primary cause of hemorrhage. These diseases furthermore demonstrate that the prothrombin of the blood can be diminished either by the action of a toxic substance or through the lack of an accessory food factor. The effectiveness of alfalfa, which seems to be rich in these vitamin-like substances, in curing both diseases is obviously of great agricultural importance. Since the hemorrhagic diathesis met with in obstructive jaundice may well be a manifestation of a vitamin deficiency, as already discussed, the handling of this condition from a dietary point of view together with the therapeutic use of bile salts is worthy of clinical trial. The observation that sweet clover alone readily produces the disease, whereas a mixture of as little as 5 per cent alfalfa with the toxic hay prevents its development, leads to the practical suggestion that sweet clover hay should never be fed alone but should always be mixed with a protective feed such as alfalfa.

With the quantitative method for the determination of prothrombin as outlined in this paper, it is possible to detect the hemorrhagic tendency in jaundice, in sweet clover intoxication, and in the chick disease long before there are significant alterations in the ordinary clotting tests or before frank signs of hemorrhage occur. By following the changes in the concentration of prothrombin quantitatively, it is possible to determine the action of the various agents employed with far greater precision than has hitherto been possible.

SUMMARY

1. A simple quantitiative method for the determination of prothrombin is presented.

2. The feeding of spoiled sweet clover hay to rabbits causes the plasma to drop to an exceedingly low level. The hemorrhagic tendency runs parallel with the reduction of prothrombin. Transfusion with citrated

blood has a marked beneficial effect, but the elevation of prothrombin is of short duration. Injection of defibrinated blood has no appreciable effect and raises plasma prothrombin only very slightly. The incorporation of 5 per cent alfalfa with the toxic hay successfully prevents the development of the disease; and a small quantity of dehydrated alfalfa meal is sufficient to cure the hemorrhagic condition by elevating the level to plasma prothrombin to within the limits of safety.

3. Chicks fed a diet (Almquist) containing all the known vitamins except the one designated by Dam as vitamin K causes a gradual but eventually a pronounced depletion of prothrombin. The addition of a small amount of alfalfa to the basal diet effectively restores the plasma prothrombin.

4. The theory is presented that spoiled sweet clover hay contains a toxic principle which either destroys prothrombin or inhibits the mechanism by which the body produces this clotting factor, and that alfalfa exerts its curative effect by supplying an accessory food factor required by the organism for the synthesis of prothrombin. This substance is not identical with vitamin K, but may be related to it since both are apparently abundantly present in alfalfa. The possibility is presented that the hemorrhagic diathesis met with in certain cases of obstructive jaundice may be due to a deficiency of the vitamin required by the body for the production of prothrombin.

REFERENCES

(1) Quick, A. J., M. Stanley-Brown and F. W. Bancroft. Am. J. Med. Sci. **190:** 501, 1935.
(2) Schofield, F. S. J. Am. Vet. Med. Assoc. **64:** 553, 1924.
(3) Roderick, L. M. and A. F. Schalk. Studies on sweet clover disease. Bull. N. D. Agric. Exp. Sta. 1931.
(4) Roderick, L. M. This Journal **96:** 413, 1931.
(5) Quick, A. J. J. Biol. Chem. Proc. **114:** lxxxii, 1936.
(6) Dam, H. and F. Schønheyder. Biochem. J. **28:** 1355, 1934.
(7) Dam, H. and F. Schønheyder. Biochem. J. **30:** 897, 1936.
(8) Schønheyder, F. Biochem. J. **30:** 890, 1936.
(9) Almquist, H. J. and E. L. R. Stokstad. J. Biol. Chem. **111:** 105, 1935.
(10) Almquist, H. J. J. Biol. Chem. **114:** 241, 1936.
(11) Dam, H., F. Schønheyder and E. Tage-Hansen. Biochem. J. **30:** 1075, 1936.
(12) Quick, A. J. This Journal **114:** 282, 1936.
(13) Warner, E. D., E. M. Brinkhous and H. P. Smith. This Journal **114:** 667, 1936.
(14) Mills, C. A. This Journal **76:** 651, 1926.
(15) Quick, A. J. This Journal **116:** 535, 1936.
(16) Dam, H. Biochem. J. **29:** 1273, 1935.
(17) Hawkins, W. B. and E. M. Brinkhous. J. Exper. Med. **63:** 795, 1936.

THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON THE EMPTYING TIME OF THE STOMACH

CLARK K. SLEETH AND EDWARD J. VAN LIERE

From the Department of Physiology, West Virginia University, Morgantown, W. Va.

Received for publication September 5, 1936

In 1929 Ivy and Fauley (1) showed that a condition of hunger decreases the emptying time of the stomach. Van Liere, Lough and Sleeth (2) found that the emptying time of the stomach is increased by administration of ephedrine. Hellebrandt (3) reported that mild exercise hastens gastric emptying, while violent and exhaustive exercise is more likely to prolong the gastric emptying time. Quigley observed that insulin increased (4) and phlorhizin inhibited (5) spontaneous gastric motility. Since it was felt that extremes of environmental temperature could simulate some of the above named conditions, and is a factor which should be controlled in all studies of gastric physiology, the present study was undertaken.

METHOD. Five healthy dogs were used in this work. They were given a standard meal at eight-thirty each morning. The meal consisted of 40 grams hamburger, 10 grams dried ground bread and 50 cc. milk. To this were added 15 grams barium sulphate, so that the position of the meal could be determined fluoroscopically. This standard meal has been used in this laboratory for a number of years, and data on the emptying time of the stomach for this meal have been obtained on a series totalling more than 50 dogs (6).

Immediately after the animals had eaten the meal they were placed in a large, well ventilated steel chamber. This chamber was located outside of the laboratory, so that the temperature within it varied directly with the temperature of the atmosphere. A number of determinations of gastric emptying time were made upon each animal at room temperature (68–75°F. or 20–26°C.).

After a constant normal figure had been established for each animal they were subjected to a cold environmental temperature by placing them in the chamber with the temperature varying between 5° and 22°F. (−15° and −5°C.) during the course of the experiment. The average temperature within the chamber for the entire experiment was 15°F. (−10°C.). This part of the work covered a period of 20 days, and no less than 10 determinations of gastric emptying time were made upon each

animal during this period. After the effect of cold environmental temperature had been definitely established the animals were given a rest of about 10 weeks, until the atmospheric temperature had become warm enough to allow continuation of the experiment. Their normal gastric emptying times were then rechecked by several determinations at room temperature and were found to have remained unchanged from the original normal figures.

The animals were then exposed to a high environmental temperature. They were placed in the same chamber, under conditions identical with those of the first part of the experiment, except that this time the average internal temperature of the chamber was 90°F. (32°C.). The extremes of variation in this set of determinations were 82°F. (28°C.) and 97°F. (36°C.) respectively. This experiment covered a period of only five days, and each animal was used every day.

TABLE 1

The effect of environmental temperature on the emptying time of the stomach

DOG NUMBER	EMPTYING TIME OF THE STOMACH					
	Normal, 68–75°F.		Cold, 15°F.		Heat, 90°F.	
	Hours	Per cent	Hours	Per cent	Hours	Per cent
1	6.37	100	5.67	89		
2	6.58	100	4.64	86	7.37	112
3	6.33	100	5.09	80	6.95	110
4	5.92	100	4.62	78	6.55	111
5	6.73	100			7.25	108
Average..	6.38	100	5.01	83	7.03	110

RESULTS. Table 1 summarizes the results obtained.

It is seen from table 1 that the exposure to cold shortened the gastric emptying time in four dogs by an average of 17 per cent, while exposure to heat delayed the gastric emptying time in the same number of animals by an average of 10 per cent. It will be noticed that animal number 1 does not appear in the series of experiments upon the effects of high temperature. This animal unfortunately met with an accident during the rest period mentioned above, and was replaced by animal number 5, which does not appear in the series of experiments upon the effect of cold.

Table 2 shows excerpts from the daily records of two characteristic animals.

The second table shows that there is a correlation between slight variations in environmental temperature and variations in the emptying time of the stomach. In virtually every case, the colder the environmental temperature, the shorter the gastric emptying time, and conversely, the

hotter the environmental temperature, the longer the time required for the stomach to empty.

Discussion. It is known that epinephrine will decrease gastro-intestinal motility by its action on the sympathetic nervous system. It has also been shown by Van Liere, Lough and Sleeth (2) that administration of ephedrine, a substance closely allied to epinephrine, delays the gastric emptying time. If we assume that cold temperatures stimulate the production of epinephrine our findings cannot be explained upon the basis of an epinephrine action, since were such the case, cold temperatures would prolong the gastric emptying time.

Although there are several mechanisms by which environmental temperature might influence the rate of gastric evacuation, we shall mention only the one which appears the most rational.

TABLE 2

Daily variation of the emptying time of the stomach, with changes in environmental temperature

DATE	TEMPERATURE	GASTRIC EMPTYING TIME	
		Dog 3	Dog 4
	°F.	hours	hours
1/24	6°	5.00	4.50
1/25	8°	5.00	4.50
2/6	11°	5.00	4.50
1/26	18°	5.25	4.75
2/5	20°	5.25	4.75
5/13	82°	6.75	6.00
3/9	85°	6.75	6.25
5/12	92°	7.25	7.00
5/18	93°	7.25	6.75
5/15	97°	7.50	7.25
Normal gastric emptying time.....		6.33	5.92

It is known that exposure to cold, or mange, with resulting increased heat loss, augments the tone and motility of the empty stomach (7). It is also known that the tone and motility of the stomach is predominantly concerned in the rate of evacuation. Thus it would appear that the factor of heat loss is of prime importance in explaining our observations. Exposure to cold, by increasing the requirement for food, operates to shorten the gastric emptying time much as a deficiency of pancreatic juice and the consequent inadequacy of digestion do (8). The opposite obtains for the delay in evacuation noted at higher temperatures, and is analogous to the depression and abolition of hunger motility by fever.

It should be noted that several determinations were made upon each animal at the cold temperatures. This was felt necessary because of the

fact that there was considerable daily variation in the temperature of the chamber. Enough determinations were made upon each animal to assure a fair average for the emptying time of the stomach at the average temperature reported.

As has been noted from previous work, the normal emptying time of the stomach of any individual animal remained strikingly constant from day to day. It is, however, worthy of particular note that the normal gastric emptying time of the animals was found to have remained at the original normal figure even after a period of 10 weeks had elapsed in the interim between the experiments on the effect of cold and of hot environmental temperatures.

A similar constancy was observed upon days of similar temperature during the course of the experiment. However, a definite, if slight, variation with relatively small changes in environmental temperature was noted. In every case, the colder the day the more rapidly the stomach would empty. The converse was also true in the case of high temperatures. These variations, although slight, were nevertheless so constant that they could not be ignored.

SUMMARY AND CONCLUSIONS

Work done upon the emptying time of the stomach in five normal dogs shows that low environmental temperature decreases the emptying time of the stomach, while high environmental temperature increases the gastric emptying time. Four animals exposed to temperatures averaging 15°F. (−10°C.) showed an average decrease of 17 per cent in the emptying time of the stomach, while the same number of animals, exposed to temperatures averaging 90°F. (32°C.) showed an average increase of 10 per cent in the emptying time of the stomach.

Some of the possible mechanisms involved are discussed in the body of the paper.

This work gives experimental evidence for the basis of the recognized fact that an individual often feels more hungry during cold weather and less hungry in extremely warm weather.

REFERENCES

(1) Ivy, A. C. and G. B. Fauley. This Journal **91**: 206, 1929.
(2) Van Liere, E. J., D. H. Lough and C. K. Sleeth. J. A. M. A. **106**: 535, 1936.
(3) Hellebrandt, F. A. and R. H. Tepper. This Journal **107**: 355, 1934.
(4) Quigley, J. P. and B. O. Barnes. This Journal **95**: 7, 1930.
(5) Quigley, J. P. and J. L. Lindquist. This Journal **92**: 690, 1930.
(6) Van Liere, E. J., G. Crisler and I. A. Wiles. J. Lab. and Clin. Med. **22**: 261, 1936.
(7) Carlson, A. J. The control of hunger in health and disease, p. 271, Chicago, 1916.
(8) Fauley, G. B. and A. C. Ivy. This Journal **89**: 428, 1929.

PITUITARY REGULATION OF WATER EXCHANGE IN THE DOG AND MONKEY

H. L. WHITE AND PETER HEINBECKER

From the Departments of Physiology and of Surgery, Washington University, St. Louis

Received for publication September 8, 1936

Teel (1929) produced polyuria in normal dogs by pituitary anterior lobe extracts. Barnes, Regan and Bueno (1933) obtained polyuria on anterior lobe administration to normal but not to thyroidectomized dogs and believed that the diuretic action of anterior lobe was due to its thyrotropic action. Biasotti (1934) obtained similar results and found that the action was present after renal denervation. Mahoney and Sheehan (1935) found that the polyuria of clipping the stalk in dogs was stopped by thyroidectomy and restored by thyroid feeding, and that the polyuria, which in non-thyroidectomized animals persisted from 2 to 8 weeks, was of only 1 to 2 days' duration when the stalk was clipped in previously thyroidectomized dogs.

Barnes, Regan and Bueno found anterior lobe administration effective in producing polyuria in thyroidectomized dogs provided they were given thyroid by mouth. This finding hardly seems compatible with their rejection of the view that the anterior lobe contains a diuretic principle and their acceptance of the alternative view that anterior lobe produces polyuria merely by stimulating the thyroid, the increased activity of the latter being responsible for the polyuria. If it can be shown that thyroid, given to thyroidectomized animals in amounts sufficient to bring on polyuria when combined with anterior lobe administration, does not, without concomitant anterior lobe administration, produce a polyuria, such a finding should favor the view that anterior lobe contains a diuretic substance. The experiments of Barnes, Regan and Bueno do not answer this point.

The present work was undertaken to answer the above point, to study the effect of thyroidectomy and thyroid administration on the polyuria of anterior lobe administration, and to compare the sensitivity of the normal and the hypophysectomized dog to the diuretic effect of anterior lobe.

METHODS. In most of the experiments the animals were on a constant diet of Purina dog chow and milk; in some a bread and meat diet was used. Twenty-four hour urine collections were made in metabolism cages; fluid

intake and urine specific gravity were also followed. The fluid intake and specific gravity findings are not shown in the charts but, as would be expected, the former paralleled the urine output and the latter fell with increased urine volume. A total of nine dogs have been followed for periods ranging from 2 to 6 months. Hypophysectomies were done by a temporal approach, the gland being removed by crushing and suction under direct inspection. In the animals which have come to autopsy, completeness of hypophysectomy has been confirmed. A marked polyuria always followed hypophysectomy, in some cases lasting only 3 or 4 days, in others 2 to 3 weeks. On thyroidectomy two parathyroids were always preserved. In only one case did tetany occur; it was manifested on the third postoperative day and was controlled by daily injections of parathyroid extract for two days.

Except for one lot of Phyone, which was kindly supplied by Dr. David Klein of the Wilson Laboratories, the anterior lobe extract used was an acid extract, prepared essentially according to Loeb and Bassett (1930). The anterior lobes were separated from the glands (beef) at the slaughter house within a few minutes of the animals' death. Fifty grams of fresh tissue were ground with a little sand and extracted for two days in the ice box in 200 cc. of 0.5 per cent acetic acid, centrifuged, neutralized, recentrifuged after 2 or 3 hours further in the ice box and finally passed through a Seitz filter. In one case it was definitely shown that an extract produced diuresis when 10 days old and was inactive when 17 days old; in general the extracts were discarded after 12 days. It was also found that separating the anterior lobes from the remainder of the glands and keeping them cold from the time of separation increased the incidence of positive results. Administration was always intraperitoneal. The extracts prepared by us were shown to contain growth hormone, as indicated by their causing resumption of growth in the hypophysectomized immature rat, and thyrotropic and gonadotropic hormone, as indicated by morphological changes in the immature female guinea pig.

Observations. 1. Daily administration on two consecutive days of 1.7 to 2 cc. per kilo of anterior lobe acid extract (0.25 gram fresh tissue per cc.) usually increases the urine output of normal dogs by 2 to 5 fold; the increase appears within 24 to 72 hours and lasts 3 to 6 days. In about half the cases there was a distinct initial antidiuretic effect lasting 1 to 3 days, which was usually followed by increased output, although in a few cases only the antidiuresis phase was present. Rarely no significant change occurs; when this is the case the sample of extract may be inactive on other dogs, although occasionally a dog which has responded to one batch of extract will fail to respond to another although the latter batch may be active with some other dog.

2. The response of the hypophysectomized dog, after recovery from the

transient polyuria, to anterior lobe extract is similar to that before hypophysectomy; the impression is given that the response is, on the average, greater than in the normal. Inability to get closely reproducible results with conditions maintained as nearly constant as possible makes it impossible to say with finality that the hypophysectomized dog is more sensitive to the diuretic action of anterior lobe than is the normal.

3. The daily administration for 6 to 8 days of 0.1 gram per kilo of desiccated thyroid (0.3 to 0.34 per cent iodine) produces no significant change in daily urine output of either the hypophysectomized or the hypophysectomized and thyroidectomized dog.

4. The daily addition for 2 days of 2 cc. per kilo of anterior lobe acid extract during a period of thyroid administration, beginning after 6 to 8 days of thyroid, usually produces in hypophysectomized dogs an increase in daily urine output about the same as on anterior lobe extract alone. Variations in individual responses are too great to permit a positive conclusion as to an augmentation by thyroid of the response to anterior lobe, although on a few occasions dogs (not thyroidectomized) which have failed to respond to anterior lobe alone have responded to anterior lobe plus thyroid.

5. Daily administration for 2 days of 2 cc. per kilo of anterior lobe extract does not produce any increase in urine output in the hypophysectomized and thyroidectomized dog within 3 weeks after thyroidectomy but may produce an increase, although smaller than in the normal or thyroid-fed animal, if given 2 months or later after thyroidectomy.

6. Daily administration for 2 days of 2 cc. per kilo of anterior lobe extract to the hypophysectomized and thyroidectomized dog during a period of thyroid administration (0.1 gram per kilo daily) shows results essentially the same as those seen in the hypophysectomized dog before thyroidectomy.

7. In a single experiment, thyroidectomy 8 days after hypophysectomy, while the polyuria of hypophysectomy was still at its height, stopped the polyuria within 24 hours, confirming Mahoney and Sheehan (1935).

8. The daily administration for 2 days of 2 cc. per kilo of anterior lobe extract to normal monkeys always produced an antidiuretic response for 1 to 2 days, followed by a variable diuretic response. With larger doses (up to 5 cc. per kilo) the antidiuretic phase was exaggerated and the diuretic diminished. In no case was the antidiuretic phase absent, even with extracts which produced only diuresis in dogs.

Evidence for the above statements is shown in the charts of figure 1; they are supported by the findings on five additional dogs and one monkey, not shown in the figure.

Discussion. The findings indicate the presence in beef anterior lobe extracts of a diuretic principle which cannot act in the absence of thyroid.

That the diuretic principle is not merely the thyrotropic hormone is indicated by the finding that with a constant intake of thyroid by mouth, insufficient in itself to produce diuresis, the thyroidectomized dog shows a diuretic response to anterior lobe administration which is ineffective without the thyroid. Whether, in the dog, thyroid provides some specific

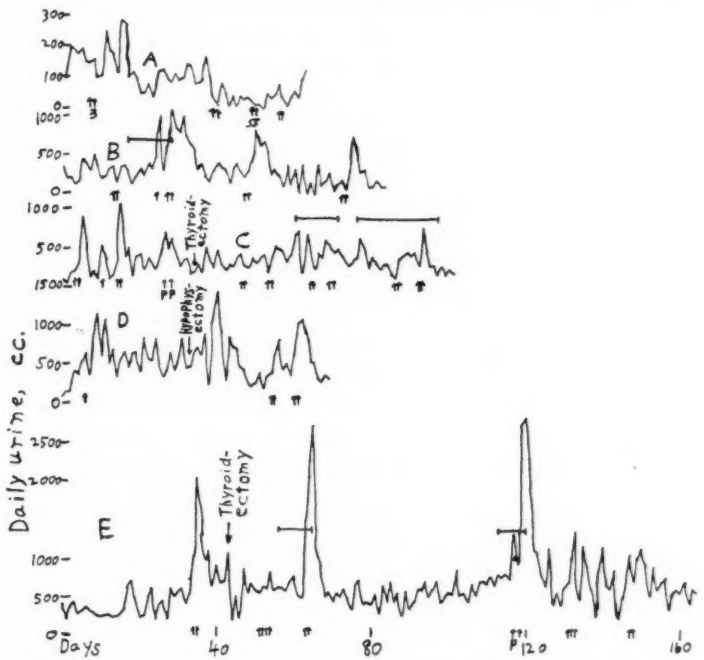


Fig. 1. |————|, desiccated thyroid, 0.1 gram per kilo. ↑, anterior lobe extract, 1.7 to 2 cc. per kilo. ↑P, Phyone. Where more than 2 cc. per kilo of extract was given the figure under the arrow designates the dose in cubic centimeter per kilo. *A*, normal female monkey; *B*, male dog, hypophysectomized 15 days before beginning of chart; *C*, male dog, hypophysectomized 3 months before beginning of chart, thyroidectomized as indicated on chart; *D*, male dog, hypophysectomized as indicated on chart; *E*, male dog, hypophysectomized 18 days before beginning of chart, thyroidectomized as indicated on chart.

agent which sensitizes the organism to the diuretic principle of anterior lobe, or whether the increase in metabolic rate is the essential condition for effectiveness of the anterior lobe principle, cannot be answered at present. The answer would involve determining whether some other agent which would increase metabolic rate would also render the thyroidectomized dog capable of responding to the anterior lobe principle.

The finding (see curve *E*) that within the first three weeks after thyroidectomy anterior lobe is without any diuretic effect, while later it may show some effect even without thyroid administration, may mean either that some hypertrophy of remaining aberrant thyroid tissue has taken place or that the organism gradually acquires the ability to respond in the absence of thyroid. Since this has occurred in previously hypophysectomized dogs the question is raised as to the possibility of compensatory hypertrophy of a remaining fragment of thyroid in the absence of the hypophysis.

No conclusive evidence has been obtained of a difference in sensitivity of the normal and the hypophysectomized dog to the diuretic effect of anterior lobe administration.

In some cases, with the thyroid gland present or thyroid given by mouth, dogs show an antidiuretic response to anterior lobe, usually followed by diuresis. A pure antidiuretic response is the usual result with both normal and hypophysectomized rats (unpublished observations) and the observations on monkeys reported here show that an antidiuretic phase is always present. The occasional finding in the dog of no change in urine output on anterior lobe administration may be due to a balance between the two effects. Whether the antidiuretic effect is due to a contamination with posterior lobe principle or to the antidiuretic anterior lobe principle described by Downes and Richards (1935) cannot be answered; great care was taken, however, to avoid contamination with posterior lobe. It appears that dogs are less sensitive than are monkeys to the antidiuretic activity of the anterior lobe preparation used.

The polyuria following hypophysectomy was suppressed immediately by thyroidectomy, confirming Mahoney and Sheehan's observations on clipping the stalk. This is of interest in view of the usual finding that the fall in basal metabolic rate does not begin until considerably more than 24 hours after thyroidectomy.

Mahoney and Sheehan (1936) have recently shown that whereas clipping of the pituitary stalk in the dog is followed by polyuria, this procedure has no effect on water exchange in the monkey. This species difference apparently has an anatomical basis in that clipping of the stalk in the monkey does not injure either hypothalamus or pituitary, while in the dog lower hypothalamic injury is unavoidable and degeneration of the anterior lobe occurs; degeneration of the posterior lobe may or may not occur. They explain their findings as to species difference in the effect on water exchange solely on the basis that hypothalamic injury occurs in the dog and not in the monkey and conclude that the hypothesis of Fisher, Ingram and Ranson is not applicable to the latter animal. The justification for this conclusion is not clear; it omits the fact that there is also a species difference as to pituitary damage, which occurs in the dog and not

in the monkey. The questions arise whether their procedure severs the supraoptic-hypophyseal tract in the monkey and whether the denervated posterior lobe in the monkey continues to function.

The finding that several months after clipping the stalk in the dog, by which time the polyuria has disappeared, the glandular portion may be found degenerated with the nervous portion apparently intact is not inconsistent with the view of Richter (1934) and of Fisher, Ingram and Ranson (1935) that anatomical or functional removal of the posterior lobe, together with maintenance of anterior lobe function, is the essential condition to polyuria. The important point is the condition of the glandular and nervous portions during the period of polyuria. There may well be a temporary derangement of posterior lobe function, while the anterior lobe damage may be of later development. The published statements of Mahoney and Sheehan do not permit a conclusion, although their finding that the growth curve of the puppy is not influenced in the first few weeks following the operation indicates that during this period the anterior lobe is still functional.

The recent report of Reichert and Dandy (1936) that postlobectomy in the dog is without effect on the water exchange, while intense, though transient, polyuria and polydipsia were produced in two of four cases of anterior lobectomy is in more direct conflict with the Richter-Ranson view. However, in view of the much greater difficulties as to precision of operation in the dog than in the rat, and in view of the finding of Pencharz, Hopper and Rynearson (1936) that anterior lobectomy in the rat causes temporary decrease in urine output, followed by return to normal, unqualified acceptance of Reichert and Dandy's findings would appear to demand histological evidence as to cleanness of separation and absence of damage to remaining parts.

A point stressed by Mahoney and Sheehan and by Reichert and Dandy as opposing the pituitary theory of diabetes insipidus is that polyuria may be obtained by hypothalamic injury in the previously hypophysectomized animal. In view of the nervous origin of the posterior lobe it may be that neighboring hypothalamic structures may participate in the production of an antidiuretic principle and that this rôle may be increased after hypophysectomy. Indeed, direct experimental evidence that this is the case in the dog is afforded by the work of Sato (1928), who found the antidiuretic action of extracts of the hypothalamus, particularly from the region of the mammillary bodies, strongly increased some weeks after hypophysectomy. The polyuria of hypothalamic injury in the previously hypophysectomized animal may then be regarded as due to injury or denervation of the accessory sites of production of the antidiuretic principle, and as comparable with that produced by posterior lobe denervation or ablation in the animal not previously operated. If Sato's work

is correct one might find that postlobectomy in the dog need not always produce permanent polyuria. A further conclusion would be that in the rat, in which postlobectomy always produces permanent polyuria, the mammillary bodies are not capable of taking over the production of antidiuretic principle; this point is susceptible to experimental investigation.

A difficulty in accepting the view of Richter and of Fisher, Ingram and Ranson still remains, if polyuria can be produced by hypothalamic injury in the previously hypophysectomized animal, in that polyuria should not occur, even if accessory production of antidiuretic principle had been established after hypophysectomy and then interfered with by subsequent hypothalamic injury, unless some anterior lobe tissue remains. It may be pointed out that the belief that polyuria can be produced by hypothalamic injury in the previously *completely* hypophysectomized animal rests on none too secure a foundation. The belief is based on three reports before 1936, Camus and Roussy (1913, 1914, 1920), Warner (1931) and Houssay, Carulla and Romana (1920). The positive findings reduce themselves to the following. In animals reported as being free from anterior lobe tissue each group of workers produced polyuria in only one case. Warner's case lasted five months, in the other two the polyuria was quite transitory. In Warner's case the polyuria did not develop until twenty days after the injury. Reichert and Dandy report a transient polyuria (apparently of 1 to 2 days' duration) on mechanical stimulation of the hypothalamus in 1 case of a series of 11 completely or partially hypophysectomized dogs. The positive case was one reported as complete but no report of microscopic verification of absence of anterior lobe tissue was made. Mahoney and Sheehan (1936) state that they have produced "severe upset of water metabolism" by hypothalamic injuries in previously hypophysectomized monkeys but give no further report of their findings. It seems justifiable to state that at the present time the evidence for the production of polyuria by hypothalamic injury in the proved absence of any anterior lobe tissue is not completely satisfying. It may be that the transient and rarely reported polyurias under these circumstances are due to vasomotor disturbances and are quite unrelated to diabetes insipidus.

On the basis of the above considerations the following view may be held on experimental diabetes insipidus. Anatomical or functional removal of the posterior lobe in the presence of functional anterior lobe always produces permanent polyuria in the rat and cat. Judgment may be reserved on the finding of Reichert and Dandy that postlobectomy in the dog does not produce even transient polyuria, pending histological evidence; if Sato's work is correct one need not, however, predict a *permanent* polyuria in the dog. Polyuria by hypothalamic injury in the previously hypophysectomized animal has been produced only rarely and inconsistently and is always of short duration. In view of the extremely low incidence

of positive results in the proved absence of anterior lobe tissue, of the possibility that such results may be due to vasomotor disturbances, and of the "spontaneous" fluctuations of urine output occasionally seen, these observations do not justify rejection of the view advanced by Richter and by Fisher, Ingram and Ranson. The production of polyuria and polydipsia by anterior lobe extracts in both normal and hypophysectomized dogs is further evidence of the positive rôle of the anterior lobe, as is the finding of Ingram and Barris (1935) that electrical stimulation of the superficial aspect of the anterior lobe in close proximity to the pars tuberalis may produce transitory diuresis in cats. The finding that anterior lobe extracts, while not effective in the absence of thyroid, are effective when accompanied by doses of thyroid insufficient in themselves to produce diuresis indicates a specific diuretic principle from the anterior lobe.

The significance of the antidiuretic action of anterior lobe extracts is not clear; it may be of physiological significance or may be due merely to products not present in the living gland. While no satisfactory explanation of the transient polyuria of complete hypophysectomy is available, the longer duration of the diuresis induced by anterior lobe administration than of the antidiuresis of posterior lobe administration suggests that it may be merely a matter of the greater stability of the diuretic than of the antidiuretic principle.

SUMMARY

1. Evidence is presented that anterior lobe contains a diuretic principle, not the thyrotropic hormone, which is ineffective in the absence of thyroid; whether thyroid is the only agent capable of sensitizing the organism to the diuretic principle has not been determined.

2. The thyroidectomized dog gradually regains the ability to show a diuretic response to anterior lobe administration; this is true even if the animal is also hypophysectomized. Whether this is due to a regeneration of remaining thyroid tissue or to an adaptation of the organism to the absence of thyroid has not been determined.

3. The daily administration for 6 to 8 days of 0.1 gram of desiccated thyroid per kilo does not change the water exchange either of hypophysectomized or of hypophysectomized and thyroidectomized dogs. Thyroidectomized and thyroidectomized-hypophysectomized dogs fail to respond to anterior lobe extracts when thyroid is not administered but when on the above dosage of thyroid they show a marked diuretic response to anterior lobe, a specific diuretic principle from the anterior lobe is indicated.

4. No conclusive difference in the sensitivity of normal and of hypophysectomized dogs to the diuretic action of anterior lobe could be dem-

onstrated, although it is our impression that the hypophysectomized animal is somewhat more sensitive.

5. The transient polyuria of hypophysectomy in the dog is stopped within 24 hours by thyroidectomy.

6. An initial antidiuretic phase of response to anterior lobe may be present in the dog; it is always present in the monkey and may be the only response.

7. It is believed that the available evidence on experimental diabetes insipidus can be reconciled with the view that a permanent polyuria requires the presence of functional anterior lobe tissue and the absence of functional posterior lobe or other tissue capable of producing the antidiuretic principle normally produced in the posterior lobe. The possibility exists that a brief polyuria may be produced by hypothalamic injury in the absence of anterior lobe tissue but such findings do not justify rejection of the above view.

REFERENCES

BARNES, B. O., J. F. REGAN AND J. G. BUENO. This Journal **105**: 559, 1933.

BIASOTTI, A. Compt. rend. Soc. Biol. **115**: 329, 1934.

CAMUS, J. AND G. ROUSSY. Compt. rend. Soc. Biol. **75**: 628, 1913.
Presse Medicale **22**: 517, 1914.
Endocrinol. **4**: 507, 1920.

DOWNES, H. R. AND L. RICHARDS. J. Biol. Chem. **110**: 81, 1935.

FISHER, C., W. R. INGRAM AND S. W. RANSON. Arch. Neurol. and Psychiat. **34**: 124, 1935.

HOUSSAY, B., J. E. CARULLA AND L. ROMANA. Compt. rend. Soc. Biol. **83**: 1250, 1920.

INGRAM, W. R. AND R. W. BARRIS. Endocrinol. **19**: 432, 1935.

LOEB, L. AND R. B. BASSETT. Proc. Soc. Exper. Biol. Med. **27**: 490, 1930.

MAHONEY, W. AND D. SHEEHAN. This Journal **112**: 250, 1935.
Brain **59**: 61, 1936.

PENCHARZ, R. I., J. HOPPER, JR. AND E. H. RYNEARSON. Proc. Soc. Exper. Biol. Med. **34**: 14, 1936.

REICHERT, F. L. AND W. E. DANDY. Bull. Johns Hopkins Hosp. **58**: 418, 1936.

RICHTER, C. P. This Journal **110**: 439, 1934.

SATO, G. Arch exper. Path. und Pharmakol. **131**: 45, 1928.

TEEL, H. M. J. A. M. A. **93**: 760, 1929.

WARNER, F. J. J. Nerv. Ment. Diseases **73**: 375, 1931.

SALT ECONOMY IN HUMID HEAT

C. DALY AND D. B. DILL

From the Fatigue Laboratory, Harvard University, Boston

Received for publication September 8, 1936

A recent study of physical activity in dry heat at Boulder City by Dill and co-workers (1933) revealed an economy of salt in the functioning of sweat glands. Sweat was found to contain less than one-quarter, sometimes less than one-tenth of the sodium chloride concentration of blood serum. This economy of salt was especially evident after acclimatization to sweating. In contrast with these findings many workers, including Adolph (1923), Talbert and Haugen (1927), and Fishberg and Bierman (1932) have reported that sweat may contain much higher concentrations of salt. However, Dill (1933) has pointed out that some of the methods employed in collecting sweat may influence the salt content. Also, the use of subjects unacclimatized to sweating will give high values for sweat chloride. In view of the conflicting data and the fact that excessive loss of sweat chloride produces heat cramps, as shown by Moss (1923) and Talbott (1935), it seemed desirable to make further observations.

Another experiment, similar to the previous investigation at Boulder City, was carried out in Boston in order to study salt losses and acclimatization in humid heat. In the latter part of June, 1933, three subjects C. D., F. C., and W. C., started on a constant diet which was maintained during both control and sweat periods. The food was obtained as nearly as possible from one lot, either canned or of such a nature that it could be kept for several months. Essentially the diet consisted of chicken, corn, unsalted potato chips, grape nuts, cheese, jelly, unsalted crackers, dried milk, and fruit. Urinary output became constant by July 8 and the interval from that date to July 16 was used as the control period. The days of the control period were spent performing light tasks in a cool basement laboratory where sweat production was minimal. The next period, July 17 to August 8, was spent in walking and playing tennis for three to four hours during the heat of the day. Since the basic diet furnished somewhat less than 3,000 calories, glucose candy, practically free of salt and nitrogen, was used to supply the additional energy required during this period of increased activity. Urine was analyzed according to the methods described in a previous paper by Dill et al. (1933). Water content of the food was determined by drying to constant weight at 100°C. Water of metabolism was calculated from data given by Rowntree (1922).

The composition of the sweat produced by the three subjects was determined by an indirect method described by Dill et al. (1933). Accuracy of this method depends upon *a*, constancy of diet; *b*, constancy of insensible cutaneous perspiration and other extra-sweat water losses; *c*, sweat volume, and *d*, duration of the experiment. By insensible cutaneous perspiration we mean the water diffusing through the skin independent of sweat gland secretion. It is generally agreed that insensible perspiration is constant in rest but the evidence is conflicting in exercise and exposure to high temperature. However, we believe that the most convincing data show a fairly steady cutaneous water loss under widely varying conditions.[1] In any event, error from this source must be small in our experiment. This is due to the short period of activity, the relatively large volume of sweat and the moderate conditions under which the sweat was secreted.

Mean values for the daily water exchange, sweat volumes, and urinary output are given in table 1. Sweat volume was obtained by subtracting the water output in urine; in insensible cutaneous perspiration, 500 to 700 cc., in expired air, 400 cc.;[2] and in feces, 100 cc.; from the total water intake. The intake includes water drunk, water content of food, and the water formed during the metabolism of protein, fat, and carbohydrate. Body weights were observed daily and the sweat volumes corrected, as shown in the table, for the changes in weight. Sweat volumes for the

[1] Both Loewy (1911), (1914) and Whitehouse (1932) believe that work and high temperature increase cutaneous water loss through the skin to several times the resting value. However, we have reason to question these conclusions. Loewy used subjects with sweat glands affected by disease and studied only a small part of the total body area. Whitehouse used normal subjects and corrected for the secretion of the sweat glands by a method based upon the assumption of a uniform concentration of sweat chloride. Such experiments are not conclusive since Dill et al. (1936) and Daly (unpublished experiments) find that the chloride content of sweat varies considerably and the amount of insensible cutaneous perspiration may be small compared to the total sweat volume. We are more inclined to accept the data of Richardson (1926) who found that the insensible cutaneous water loss in an individual with congenital absence of sweat glands did not rise above 33 grams per hour in light exercise and high temperature. Whitehouse (1932) explains Richardson's results on the basis of a defect in skin circulation, but there is no evidence that the subject was deficient in this respect. As a matter of fact, there is good reason to believe that the blood flow in the skin was quite efficient. The subject was able to keep his body temperature within safe limits even though his metabolism increased 250 per cent. In another experiment the subject maintained a normal temperature when exposed to hot air at 50°C., by the simple expedient of wetting his underclothing. It would seem unlikely that the subject could control his body temperature under the above conditions if his skin circulation were deficient.

[2] In a previous report by Dill et al. (1933) partition of the insensible water loss between skin and lungs was given as 800 and 100 cc., respectively. The values should be about 500 to 700 cc. for skin and 400 cc. for lungs.

TABLE 1

Mean daily water exchange and urinary output in humid heat

SUBJECT	PERIOD	WEIGHT CHANGE	TOTAL FLUIDS	SWEAT VOLUME*	URINARY OUTPUT				
					Water	Chloride	Sodium	Potassium	Nitrogen
		kgm.	*liters*	*liters*	*liters*	*m.-eq.*	*m.-eq.*	*m.-eq.*	*gm.*
C. D.	July 8–16	+0.05	2.76	0.29	1.20	147	139	82	8.73
	July 17–Aug. 8	−0.09	5.74	3.66	0.95	94	93	68	7.57
F. C.	July 8–16	−0.10	2.44	0.52	0.80	138	131	77	9.58
	July 17–Aug. 8	−0.14	5.57	3.36	1.13	87	84	67	8.67
W. C.	July 8–16	+0.09	2.12	0.15	0.84	147	128	82	9.08
	July 17–Aug. 8	−0.10	4.65	2.79	0.94	84	71	73	8.10

* Due to a smaller surface area, 500 cc. were used for the cutaneous water loss of W. C.

TABLE 2

Mean daily composition of sweat in humid heat

PERIOD	JULY 17–19	JULY 24–27*	JULY 28–30	JULY 31–AUG. 2	AUG. 3–5	AUG. 6–8	MEAN VALUES	
							July 17–27	July 28–Aug. 8
C. D.								
Water, liters	3.18	3.62	4.54	3.80	3.05	3.73	3.40	3.78
Cl, *m.-eq. per l*	26	24	12	10	17	13	25	13
Na, *m.-eq. per l*	23	23	11	5	13	11	23	10
K, *m.-eq. per l*	8	2.5	2.1	5.2	4.8	2.2	5.3	3.6
N, *mgm. per l*	162	200	445	479	261	479	181	416
F. C.								
Water, liters	2.77	3.25	4.45	4.39	2.56	3.01	3.01	3.6
Cl, *m.-eq. per l*	23	29	17	13	12	15	26	14
Na, *m.-eq. per l*	27	24	18	11	10	13	26	13
K, *m.-eq. per l*	0	5.2	2.3	2.9	7.5	3.7	2.6	4.1
N, *mgm. per l*	518	133	246	409	333	305	326	331
W. C.								
Water, liters	2.60	2.25	3.49	3.79	2.38	2.31	2.43	2.99
Cl, *m.-eq. per l*	32	30	25	22	16	21	31	21
Na, *m.-eq. per l*	35	27	24	19	8	17	31	17
K, *m.-eq. per l*	2.9	1	0.9	5.3	6.4	4.3	2.0	4.2
N, *mgm. per l*				236	422	710		456

* The period July 20–23 was omitted because a satisfactory sweat volume could not be obtained on account of the prevailing low temperature.

three subjects varied from 0.15 to 0.52 liter during the control period and from 2.79 to 3.66 liters during the sweat period. The urinary output of chloride, sodium, potassium, and nitrogen from July 17 to August 8 was considerably lower than during the control period. This is the result of extra-renal losses through the sweat glands. The difference between the urinary output of a substance during control and sweat periods divided by the difference in sweat volumes for the two periods gives the concentration of the substance per unit volume of sweat.

Average values for the concentration of salt and nitrogen in the sweat are given in table 2. One of the most striking features is the decrease in sodium and chloride concentration after the first six days of the work period. During acclimatization both sodium and chloride averaged 27 m.-eq. per liter of sweat. After acclimatization the sodium and chloride concentrations fell to mean values of 13 m.-eq. and 16 m.-eq. per liter, respectively. Potassium and nitrogen did not show this type of response to continued sweating. Hancock (1929) believes that the potassium found in sweat and sweat washings comes largely from contamination by sebaceous secretions and skin epithelium. However, our data, averaging 4 m.-eq. per liter, were obtained by indirect analysis, thus indicating that potassium may be a normal component of sweat. The nitrogen varied from 200 to 700 mgm. per liter, the mean concentration being about twice that of blood urea nitrogen. The data also show individual variation. Sweat secreted by W. C. was definitely more concentrated than that of C. D. or F. C.

The above data are quite comparable to those obtained at Boulder City by Dill et al. (1933). Subjects D. D., H. E., F. C. and W. C. at Boulder City had a sweat composition very similar to that of C. D., F. C. and W. C. at Boston. Two other subjects, S. A. and B. J., secreted more dilute sweat than any of the other subjects either at Boulder City or in Boston. Only subjects F. C. and W. C. took part in both experiments. The salt content of their sweat after adaptation to sweating is of the same order of magnitude in the two experiments. Because these two men had already been sweating before starting on the experiment at Boulder City, a comparison during the period of acclimatization could not be obtained. The average daily maximum temperature during the Boulder City experiments was around 105°F. with a relative humidity of 20 per cent, while the Boston experiments were carried out at an average daily maximum temperature of 83°F. and a relative humidity of 78 per cent during the heat of the day. The Boston experiments not only confirm the findings at Boulder City, but show more clearly the acclimatization which occurs during the first week of active sweating. This initial change in salt concentration during continued sweating makes it difficult to evaluate data obtained from a few short experiments on individuals in an unknown state of acclimatization.

SUMMARY

The salt content of sweat produced by three subjects during moderate work under natural sweating conditions was studied for three weeks in the moist summer heat of Boston. Maximum daily temperatures averaged 83°F. with an average relative humidity of 78 per cent. For the first six days of work in humid heat, during acclimatization, the concentration of chloride in sweat averaged 27 m.-eq. per liter. During the following twelve days of work, after acclimatization, sweat chloride dropped to 17 m.-eq. per liter. Sweat sodium closely followed the changes in chloride concentration while potassium and nitrogen failed to show significant variations. Our experiments indicate that sweat secreted in moderate activity at moderate temperatures may contain less salt than sweat produced under extreme laboratory and industrial conditions.

The authors wish to express their appreciation for the coöperation of Messrs. Frank and Will Consolazio as subjects in this experiment.

REFERENCES

ADOLPH, E. F. This Journal **66**: 445, 1923.
DILL, D. B., C. DALY AND A. V. BOCK. J. Biol. Chem. **114**: Proc. xxv, 1936.
DILL, D. B., B. F. JONES, H. T. EDWARDS AND S. A. OBERG. J. Biol. Chem. **100**: 755, 1933.
FISHBERG, E. H. AND W. BIERMAN. J. Biol. Chem. **97**: 433, 1932.
HANCOCK, W., A. G. R. WHITEHOUSE AND J. S. HALDANE. Proc. Roy. Soc. London, Series **B105**: 43, 1929–30.
LOEWY, A. AND W. WECHSELMANN. Arch. Path. Anat. **206**: 79, 1911.
LOEWY, A. Biochem. Ztschr. **67**: 243, 1914.
MOSHER, H. H. J. Biol. Chem. **99**: 781, 1933.
MOSS, K. N. Proc. Roy. Soc. London, Series **B95**: 181, 1923–24.
RICHARDSON, H. B. J. Biol. Chem. **67**: 397, 1926.
ROWNTREE, L. G. Physiol. Rev. **2**: 116, 1922.
TALBERT, G. A. AND C. O. HAUGEN. This Journal **81**: 74, 1927.
TALBOTT, J. H. Medicine **14**: 323, 1935.
WHITEHOUSE, A. G. R. Proc. Roy. Soc. London, Series **B117**: 139, 1935.
WHITEHOUSE, A. G. R., W. HANCOCK AND J. B. S. HALDANE. Proc. Roy. Soc. London, Series **B111**: 412, 1932.

THE ANTIKETOGENIC ACTION OF GLUCOSE IN THE ABSENCE OF INSULIN[1]

I. ARTHUR MIRSKY, JOSEPH D. HEIMAN AND R. H. BROH-KAHN

From the Department of Metabolism and Endocrinology, Institute for Medical Research, Jewish Hospital, Cincinnati, Ohio

Received for publication September 10, 1936

The administration of carbohydrate or other glycogenic metabolites to fasting, or diabetic patients or to phlorhizinized dogs (1) abolishes the production of ketone bodies. Chaikoff and Weber (2) and Selle (3) could not obtain a similar effect in the fasted, depancreatized dog and concluded that the antiketogenic action of carbohydrate does not occur in the absence of insulin.

More recently we presented evidence which led us to postulate that the antiketogenic action of insulin is due to its antiglycogenolytic effect (4). Accordingly, any process which will favor the retention of glycogen in the liver of the fasting, depancreatized dog receiving no insulin should also prevent ketosis.

Since glycogen formation from glucose can occur in the liver of the completely depancreatized dog without the intervention of insulin (5) (6), we set out to test our hypothesis by studying the effect of the repeated, intravenous injection of large amounts of glucose on the blood and urinary ketones of the fasted, depancreatized dog in the absence of insulin. If insulin is essential for the antiketogenic action of glucose, then the administration of even large amounts of the latter should be ineffective in abolishing the ketosis of such animals. However, if the presence of glycogen in the liver is the essential factor in the antiketogenesis, the administration of glucose alone should be effective in this regard.

METHODS. These studies were performed on 28 dogs from which the pancreas was removed carefully and completely 96 hours before the actual experiment. During the interval between the operation and the experimental period, no food or insulin was administered. The effect of glucose administration was observed in two groups of depancreatized dogs. The first group consisted of *a*, animals from which the kidneys were removed under sodium amytal[2] anesthesia immediately before the experiment, and *b*, unanesthetized animals in which the bladders were

[1] Aided by the David May Memorial Fund.

[2] We are indebted to Eli Lilly Co. for generous supplies of sodium amytal.

catheterized and urine samples collected. This latter group enabled us to eliminate any effect of general anesthesia. The second group consisted of dogs to which one gram of phlorhizin in oil and one gram of phlorhizin in sodium carbonate solution were injected subcutaneously 72 hours after the pancreatectomy, the actual experiment being started 24 hours later.

Where the sodium amytal anesthesia was not employed, novocaine was used locally; and, in all instances, the femoral artery and vein were exposed. Arterial blood samples were drawn two hours and immediately before, and at two-hourly intervals after the injection of glucose. The "total acetone" content of the blood was determined by the Van Slyke and Fitz method, the blood sugar by the Somogyi modification of the Shaffer-Hartmann method, and the total solids by drying a weighed quantity of blood to constant weight. Where the kidneys remained intact, the bladder was emptied and urine collected for two hours before and at two-hourly intervals after the injection of glucose, and the ketone content determined by the Van Slyke and Fitz method.

TABLE 1

TYPE OF EXPERIMENT	BLOOD ACETONE				URINE ACETONE		
	Before glucose		After glucose		Before glucose	After glucose	
	2 hrs.	0	2 hrs.	4 hrs.	2 to 0 hrs.	0 to 2 hrs.	2 to 4 hrs.
	mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm.*	*mgm.*	*mgm.*
Nephrectomized-depancreatized.....	41.6	47.7	16.9	3.7			
Phlorhizinized-depancreatized.....	47.0	44.6	24.8	2.0	427	155	86

One gram of glucose per kilogram of body weight was injected into the femoral vein every half-hour in a 33 per cent solution. For control purposes, the effect of an isosmotic solution of sodium chloride was studied in a number of animals.

In all instances, the sugar and total acetone bodies of the blood were corrected for changes in the water content from the initial control period.

RESULTS. Although all our animals were treated in exactly the same manner prior to and after pancreatectomy, we found it impossible to predict the degree of ketonemia that would be found by the fourth day after the operation. The only correlation that we could make in the majority of instances was that generally a lower blood sugar (below 300 mgm. per cent) occurred in the depancreatized dogs with a severe ketosis than in those with a mild ketosis. The majority of our depancreatized animals fell into the second group.

The effect of glucose administration to anesthetized, nephrectomized-

depancreatized dogs is illustrated by the typical experiment in table 1. The antiketogenic action of glucose in the catheterized, depancreatized dog is graphically depicted by the illustration in figure 1. It is to be noted that this antiketogenic effect is the same in both groups of animals. These two sets of experiments definitely exclude the possibility of a "washing out" effect consequent to the diuretic action of glucose. It is also important to note the resumption of ketosis after the administration of glucose was stopped.

The administration of phlorhizin to depancreatized dogs hastens the onset and aggravates the severity of the ketosis. Thus a negligible keto-

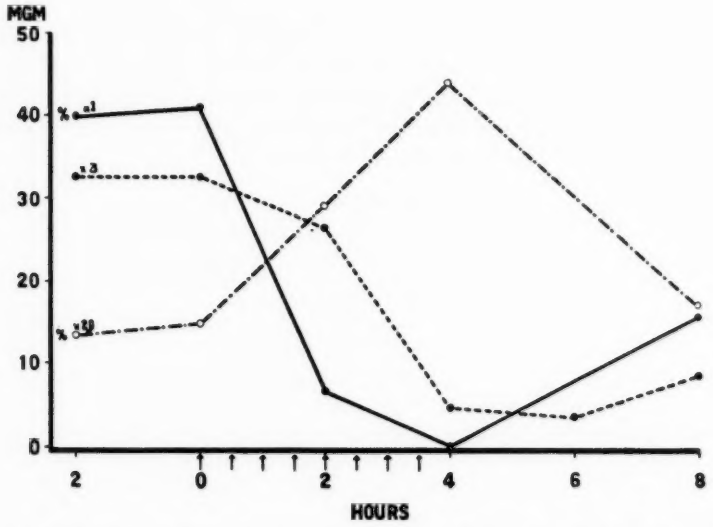


Fig. 1. The effect of the intravenous injection of glucose every half hour (↑) on the blood sugar (○— - —○), blood ketones (●———●) and urinary ketones (●--------●) of the fasted, depancreatized dog.

nemia is observed after phlorhizination of a normal dog fasted 72 hours. However, eight hours after the administration of this drug to a depancreatized dog with a mild ketonemia (11.1 mgm. per cent), the blood sugar had attained a minimum constant level (40–60 mgm. per cent) and the ketonemia correspondingly had increased (32 mgm. per cent). The intravenous administration of glucose to such depancreatized dogs always resulted in a diminution of the blood and urinary ketones just as in the depancreatized dogs which had not received phlorhizin (table 1).

Because of the obvious inverse relationship between the blood sugar and the total ketone content of the blood another set of phlorhizinized-depan-

creatized animals was treated with dextrose as in the previous experiments and the blood sugar subsequently allowed to return to the initial level. On the following day, the blood and urinary ketones were again determined before and after the injection of glucose. Such an experiment is illustrated in figure 2 which is depicted to demonstrate the specificity of glucose as well as the above mentioned inverse relationship. It may be seen that the injection of a solution of sodium chloride which was isosmotic with the 33 per cent glucose solution produced insignificant changes.

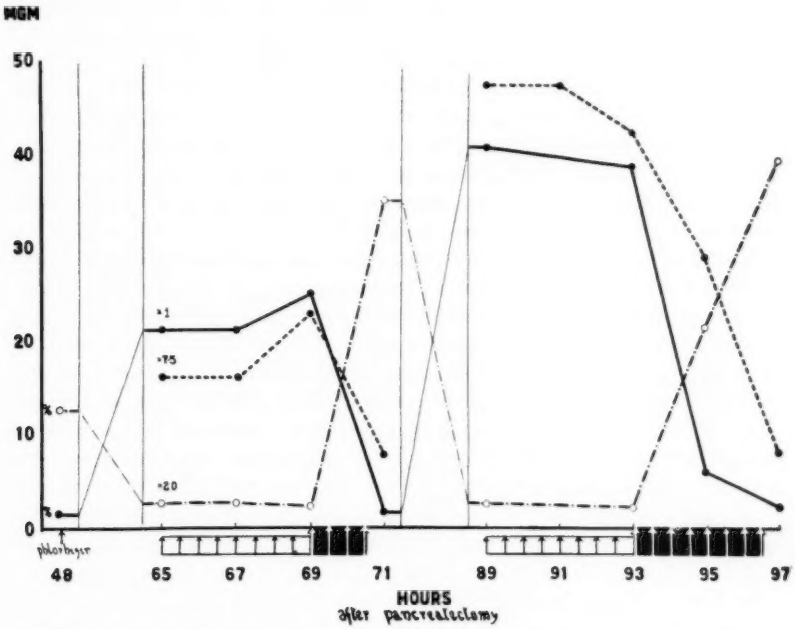


Fig. 2. Graphic illustration of the inverse relationship between the blood sugar (○— - —○) and the ketone content of the blood (●————●) and urine (●--------●) of the phlorhizinized-depancreatized dog. Black arrows indicate injection of a sodium chloride solution which is isosmotic with the glucose solution (white arrows).

It may be noted that there is no absolute relationship between the ketone content of the blood and the amount of ketones excreted in the urine. Thus, in one animal with a blood ketone content of 41.0 mgm. per cent, the urinary excretion was 377 mgm. for two hours. In another animal, with the same level of blood acetone bodies, the urinary excretion for two hours was 98 mgm.

Discussion. In a series of depancreatized dogs maintained on an undernutrition high protein diet without insulin, Soskin (7) noted a spon-

taneous diminution in the urinary ketones. The administration of glucose per os to such undernourished animals occasionally resulted in a further diminution in the ketonuria. Our data are in agreement with these observations and further make it obvious that glucose "per se" exerts a marked antiketogenic effect in the completely depancreatized dog when it is administered in amounts adequate for glycogen retention in the liver. This action may be due to *a*, an increase in the rate of ketone utilization, or *b*, a decrease in the rate of ketone formation. It is improbable that the former is the responsible factor because we have recently observed that glucose administration *per se* does not affect the rate of ketone utilization by the extrahepatic tissues (8) just as Friedemann, Somogyi and Webb (9) reported for normal animals. Since the liver is the main, if not the only site of ketone formation (10) (11) (12), and since our previous studies on the antiketogenic action of insulin (4), and our present observations on this action of glucose indicate that the disappearance of ketones from the blood of the depancreatized dog is related to the retention of glycogen in the liver, it is probable that the effects noted in these studies are due to a cessation of ketone formation

The balance between the synthesis and breakdown of glycogen in the liver of the untreated depancreatized dog is in favor of the latter so that a rapid disappearance of glycogen occurs (5) (6). When large amounts of glucose are administered parenterally, as in our experiments, there is a marked increase in the concentration of glucose in the blood (and tissues) and the balance is shifted to favor a retention of glycogen. Thus the balance between glycogenesis and glycogenolysis varies with the concentration of the free sugar in the tissues, the blood sugar level being a criterion of this balance. The fact that ketosis occurs in the fasted, depancreatized dog coincident with the decrease of liver glycogen below a certain level (which may be about 0.3 per cent) (13), and our evidence that the ketosis of such an animal can be inhibited by raising the concentration of the blood sugar, favors the existence of a critical level (or "threshold") of blood sugar above which glycogen retention occurs and ketone formation ceases, even in the absence of insulin. This concept is supported by the fact that the hypoglycemia which follows phlorhizination of the depancreatized dog is associated with an increase in the ketonemia and that an inverse relationship exists between the blood sugar and blood ketones (figs. 1 and 2). Such facts lend significance to our observations that those dogs which do not develop a severe ketosis by the fourth day after pancreatectomy and fasting have relatively high blood sugar levels.

In other conditions in which ketosis occurs such as "hunger diabetes," phlorhizin diabetes and hyperthyroidism, in all of which the insulinogenic mechanism is intact, there is no primary disturbance in hepatic glycogenolysis. In these conditions, the administration of relatively small

amounts of carbohydrate results in a deposition and retention of glycogen in the liver and a subsequent disappearance of ketosis. Since, in the absence of the pancreas a relatively larger and continuous injection of glucose alone is sufficient to produce the same effect, it becomes obvious that *the only difference between pancreatic diabetes and other conditions with ketonemia is a quantitative rather than a qualitative one.*

The data which we have discussed are in accord with our working hypothesis, that the production of ketosis is always due to a decrease in the amount of glycogen available for utilization by the liver itself (4) (10) (14). The compensatory acceleration of fat and protein catabolism which then occurs in this organ results in the gluconeogenesis which is known to take place in such conditions (15) (16) (17). Our data, also, are in agreement with the observations of Shapiro (18) which indicate that in the intact animal glycogen formation is an obligatory step in the oxidation of carbohydate and the prevention of ketosis. They lend support to the theory that the disturbance in carbohydrate, fat and protein metabolism which occurs in pancreatic diabetes is hepatic in origin and that there is no essential disturbance in the utilization of carbohydrate by the extrahepatic tissues.

SUMMARY AND CONCLUSIONS

1. The intravenous injection of large amounts of glucose to anesthetized, nephrectomized-depancreatized dogs, to unanesthetized depancreatized dogs and to phlorhizinized-depancreatized dogs, in all of which the presence of insulin was entirely excluded, results in a disappearance of ketone bodies. This antiketogenic action of glucose is attributed to the deposition of glycogen in the liver and a consequent cessation of ketone formation.

2. The administration of phlorhizin to the fasted, depancreatized dog results in a rapid drop in the blood sugar and a concomitant aggravation of the ketonemia.

3. An inverse relationship exists between the sugar and ketone bodies of the blood.

4. The degree of ketonuria is no index of the ketone body content of the blood, although a change in the latter produces a similar change in the former.

5. These studies suggest that the metabolic disturbance in pancreatic diabetes is due to an alteration in the balance between hepatic glycogenesis and glycogenolysis. The associated production of ketone bodies is attributed to a compensatory acceleration in the catabolism of fatty acids and proteins in the liver in consequence of a decrease in the amount of carbohydrate available for oxidation by the liver *per se*.

We are indebted to Miss Dorothea Hamm and Mr. Joseph Ransohoff for techical assistance.

REFERENCES

(1) WIERZUCHOWSKI, M. J. Biol. Chem. **73:** 417, 1927.
(2) CHAIKOFF, I. L. AND J. J. WEBER. Proc. Soc. Exper. Biol. and Med. **25:** 212, 1927.
(3) SELLE, W. A. Ibid. **25:** 219, 1927.
(4) MIRSKY, I. A. This Journal **116:** 322, 1936.
(5) MAJOR, S. G. AND F. C. MANN. Ibid. **102:** 409, 1932.
(6) BODO, R. C., F. C. CO TUI AND L. FARBER. Ibid. **103:** 18, 1933.
(7) SOSKIN, S. J. Nutrition **3:** 99, 1930.
(8) MIRSKY, I. A. AND R. H. BROH-KAHN. (In preparation.)
(9) FRIEDEMANN, T. E., M. SOMOGYI AND P. K. WEBB. J. Biol. Chem. **67:** xlv, 1936.
(10) MIRSKY, I. A. This Journal **115:** 424, 1936.
(11) GREENBERG, M. M. J. Biol. Chem. **112:** 431, 1936.
(12) CHAIKOFF, I. L. AND S. SOSKIN. This Journal **87:** 58, 1928–29.
(13) CHAIKOFF, I. L. J. Biol. Chem. **74:** 203, 1927.
(14) MIRSKY, I. A. AND R. H. BROH-KAHN. This Journal **117:** 6, 1936.
(15) SOSKIN, S. AND I. A. MIRSKY. This Journal **114:** 106, 1935.
(16) SOSKIN, S., I. A. MIRSKY, L. M. ZIMMERMAN AND N. CROHN. Ibid. **114:** 110, 1935.
(17) YOUNG, F. G. J. Physiol. **87:** P6, 1936.
(18) SHAPIRO, I. J. Biol. Chem. **108:** 373, 1935.

THE RESPIRATORY QUOTIENT OF RENAL TISSUE OF HOUSSAY DOGS

J. F. FAZEKAS, E. H. CAMPBELL, JR. AND H. E. HIMWICH

From the Departments of Physiology and Pharmacology and Surgery, Albany Medical College, Albany, N. Y.

Received for publication September 11, 1936

The cause for the remarkable alleviation of experimental diabetes by hypophysectomy (Houssay and Biasotti, 1931) is being intensively studied. Houssay and his co-workers concluded that the depancreatized, hypophysectomized, i.e., Houssay, animal can oxidize carbohydrate, since these observers noted a rise in the R.Q. after the ingestion of glucose. Because of the importance of this problem, it seemed advisable to obtain additional data by a different technique and therefore the respiratory metabolism of renal tissues excised from Houssay animals was determined.

Previous work on the kidney discloses that its R.Q. is raised by glucose in the normal but not in the depancreatized animal. In well fed, normal dogs Richardson, Shorr and Loebel (1930) observed an average R.Q. of 0.81 for renal tissues with no substrate present in the medium. The R.Q. was raised to 0.87 by glucose and to 0.93 by lactate. In their fasted dogs these workers observed lower quotients, 0.76 with no substrate and 0.81 with glucose. These results are in agreement with our own obtained on fasted rats (unpublished data) in which the average R.Q. was 0.74 and rose to 0.79 with glucose. On fed rats, however, Dickens and Simer (1930) noted an average quotient of 0.85 with glucose in the suspending medium. Thus, both in fasted and in fed animals, glucose consistently raises the R.Q. of the kidney.

With renal tissues of depancreatized dogs, lactate increases the R.Q. but glucose no longer does so. Richardson, Shorr and Loebel find a R.Q. of 0.71 in a non-nutrient medium, 0.72 in the presence of glucose, and 0.75 with lactate; the increase with lactate only being significant. We have also observed quotients of kidneys of depancreatized dogs close to 0.70 both with or without glucose (Himwich, Goldfarb, and Fazekas, 1936).

For the present investigations two dogs were hypophysectomized and subsequently depancreatized.[1] After an interval of approximately one

[1] Subsequent necropsy examinations of both animals disclosed no gross evidence of pituitary tissue. Several microscopic sections of the base of the brain of dog B, however, revealed a small group of cells, measuring approximately 1.0×0.6 mm. in its greatest diameters, closely adherent to the tuber cinereum. These cells resembled those of pars tuberalis.

and one-half months, their kidneys were excised and the respiratory metabolism of this tissue was determined in the Warburg apparatus.

RESULTS. The kidneys excised from dogs A and B yielded no evidence of oxidation of glucose. As the R.Q. indicated no combustion of carbohydrate, these results with the Houssay dog are in contrast to those obtained with normal animals but are similar to those observed on depancreatized dogs. On the other hand, lactate is oxidized by renal tissue excised from Houssay animals, this response to lactate being similar to that of kidney from the normal or the depancreatized animals.

TABLE 1

R.Q. of renal tissue of Houssay dogs

	NO SUBSTRATE	GLUCOSE	LACTATE	REMARKS
Dog A		0.67		Feb. 7, 1936, hypophysectomy
		0.66		Apr. 28, 1936, pancreatectomy
		0.68		June 19, 1936, blood sugar fasting
		0.670*		68 mgm. per cent, kidney excised
Dog B	0.68	0.74	0.74	May 12, 1936, hypophysectomy
Left kidney	0.72	0.70	0.76	June 18, 1936, pancreatectomy
	0.64	0.70	0.83	July 27, 1936, kidney excised
	0.680*	0.71	0.777*	
		0.71		
		0.72		
		0.713*		
Right kidney	0.68	0.65	0.78	Aug. 1, 1936, blood sugar not fasting
	0.71	0.73	0.80	344 mgm. per cent, kidney excised
	0.69	0.73	0.790*	
	0.693*	0.68		
		0.74		
		0.70		
		0.705*		

* Average.

These observations, therefore, disclose no fundamental change in the metabolism of carbohydrate as a result of combined hypophysectomy and pancreatectomy. In contrast with kidney, muscle excised from Houssay animals acts differently from that of depancreatized dogs. Shorr, Richardson and Sweet (1936) note a R.Q. of 0.73 with muscle of depancreatized dogs in a non-nutrient medium and 0.80 from that of the Houssay animal. In both cases, lactate raises the R.Q. However, observations with lactate yield no crucial evidence as to the ability of tissue to oxidize glucose.

It is interesting that the renal tissues of dog A with the low level of

blood sugar and those of dog B with marked hyperglycemia were both incapable of oxidizing glucose.

The apparently contradictory results of the intact animal and excised renal tissue might be imputed to the difference in the preparation. The R.Q. of the intact animal is a resultant of those of all the organs of the body; for example, in the depancreatized dog the R.Q. of the brain remains at unity (Himwich, Nahum, 1932) despite an R.Q. of 0.70 for the entire animal. Furthermore, a study of excised tissue excludes many factors involving intermediary changes of carbohydrate metabolism and acid-base equilibrium which might influence the R.Q. in the intact animal.

CONCLUSION

The respiratory metabolism of 3 kidneys excised from 2 Houssay animals was studied in the Warburg apparatus.

Six observations were made in a non-nutrient medium, 15 with glucose, and 5 with lactate.

Although lactate was oxidized, no evidence for the combustion of glucose was observed in these experiments.

REFERENCES

DICKENS, F. AND F. SIMER. Biochem. J. **24**: 1301, 1930.
HIMWICH, H. E., W. GOLDFARB AND J. F. FAZEKAS. This Journal **114**: 273, 1936.
HIMWICH, H. E. AND L. H. NAHUM. This Journal **101**: 446, 1932.
HOUSSAY, B. A. AND A. BIASOTTI. Endocrinol. **15**: 511, 1931.
RICHARDSON, H. B., E. SHORR AND R. O. LOEBEL. J. Biol. Chem. **86**: 551, 1930.
SHORR, E., H. B. RICHARDSON AND J. E. SWEET. This Journal **116**: 142, 1936.

THE BLOOD SUGAR LEVEL AFTER ADMINISTRATION OF PHYSOSTIGMINE AND ATROPINE

M. CAROLINE HRUBETZ

From the Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City

Received for publication September 14, 1936

In a previous publication (1) the author hoped to throw some light upon the nervous factors involved in the control of the sugar in the blood. It was found that pilocarpine raised the blood sugar level and that atropine lowered it. Atropine also prevented the rise following pilocarpine. Acetylcholine on the other hand, lowered the sugar level. The surprising disagreement between acetylcholine and pilocarpine in their effects upon the blood sugar rendered premature any conclusion as to the innervation of the islets. In the face of these results it was thought expedient to try another of the so-called parasympathetic drugs in the expectation of throwing further light on the subject.

Physostigmine (eserine) salicylate, freshly prepared each day, was used subcutaneously. All experiments were made on normal-fed rats. Approximately 50 observations were made for each point on the curves. The sugar was determined by the Somogyi micro method for blood (2). The same general plan of experimentation was employed as was followed in the earlier experiment, i.e., the effect of the physostigmine on the blood sugar level was determined before and after the administration of atropine.

PHYSOSTIGMINE. Physostigmine (eserine) salicylate was used in doses of 0.25 mgm. per kilogram. Figure 1 shows a significant rise in the blood sugar level in one hour with a return to the original level in 2 hours.

Physostigmine after atropine. As has already been pointed out (1), there is no demonstrable effect of atropine during the first half hour. On the basis of this, rats were injected with atropine (20 mgm. per kilogram) and after half an hour were injected with physostigmine in the dosage already indicated. Figure B shows that physostigmine did not raise the blood sugar after atropine.

CONCLUSION. The results are in agreement with those obtained with pilocarpine but again there is the disagreement with acetylcholine. It would seem perhaps that pilocarpine and physostigmine act by a similar mechanism which is undoubtedly different from that of acetylcholine.

Stewart and Rogoff (3) reported that the administration of physostigmine, either intravenously or subcutaneously, caused, in cats, an augmentation of the epinephrine output. Cannon and Cattell (4) suggested that atropine paralyzes the medullary cells of the adrenal glands. Since the results of this work are closely similar to those obtained with pilocarpine, the same suggestions are offered as a possible explanation of the effects. If atropine paralyzes and physostigmine stimulates the adrenal medulla, these results may be explained by assuming a disbalance of insulin and epinephrine with a normal supply of insulin in both cases. Thus, following physostigmine, there is an excess of epinephrine as compared with the amount of insulin present and with atropine, a relative epinephrine

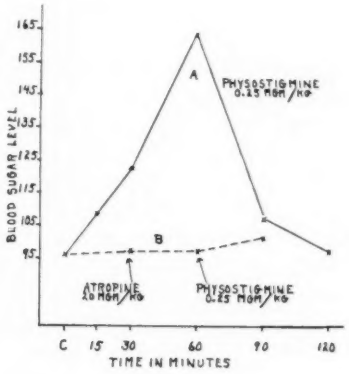


Fig. 1

insufficiency. It is also possible that these drugs do have their usual parasympathetic effects which, in turn, may be masked by the direct action upon the adrenal medulla.

SUMMARY

1. Physostigmine raises the blood sugar level. The maximum effect is produced in one hour with a return to the original level in 2 hours.

2. Atropine prevents the hyperglycemia after physostigmine.

REFERENCES

(1) Hrubetz, M. C. This Journal **114**: 551, 1936.
(2) Somogyi, M. J. Biol. Chem. **70**: 599, 1926.
(3) Stewart, G. N. and J. M. Rogoff. J. Pharmacol. Exper. Therap. **17**: 22, 1921.
(4) Cannon, W. B. and McK. Cattell. This Journal **41**: 39, 1916.

THE EFFECT OF CORTIN ON THE CONCENTRATIONS OF SOME CONSTITUENTS OF THE BLOOD OF ADRENALECTOMIZED RATS

DWIGHT J. INGLE, HUGO W. NILSON[1] AND EDWARD C. KENDALL

From the Section on Biochemistry, The Mayo Foundation, Rochester, Minnesota

Received for publication September 14, 1936

In adrenal insufficiency, as studied under the usual experimental conditions, the concentration of sodium and chloride in the serum is less than normal and it is clearly established that there is a negative balance between the intake of these ions and their output in the urine. It has been suggested by Loeb, Atchley and Stahl (1) and by Harrop and his associates (2) that this loss of sodium and chloride from the blood is the fundamental physiologic change in adrenal insufficiency and that the essential action of cortin is on the kidneys. Ingle and Kendall (3) have demonstrated the action of cortin in adrenalectomized rats which also had been nephrectomized. The object of the present investigation was to throw light on the changes in the concentrations of the principal crystalloids in the blood, and especially, to dissociate these changes from the activities of the kidneys. To this end the effect of cortin on the concentration of sodium, potassium, chloride, sugar and urea in the serum was determined for adrenalectomized rats which also had been nephrectomized or which had received intraperitoneal injections of large amounts of water.

METHODS. Male rats of the Wistar strain, which were uniform as to age and which weighed between 180 and 190 grams, were used in these experiments. All operations were done while the rats were under ether anesthesia. The kidneys and adrenal glands were removed in single stage, nonaseptic operations by the usual lumbar approach. Hemorrhage was constantly avoided.

In the work tests, the animals were anesthetized with phenobarbital-sodium. The gastrocnemius muscle was loaded with 100 grams and forced to contract three times per second by direct faradic stimulation by means of silver needle electrodes. Each stimulus consisted of both make and break shocks and was of optimal intensity. A maximum of eight animals could be used simultaneously. The electrodes to each were connected in series and the same shock stimulated all of the animals. The height of

[1] National Research Council Fellow in the Biological Sciences at The Mayo Foundation.

each muscular contraction was registered automatically. The animals were enclosed in a cabinet with the temperature constant at 28°C. The initial rate of work per minute was noted and the animals were closely observed until the untreated animals lost muscular responsiveness. (See reference 3 for complete description of apparatus and methods.)

Mixed blood was used for all the analyses carried out in these experiments. Surgical anesthesia was produced with ether before the specimens of blood were taken for the determination of sodium, chloride and potassium. The heart was exposed and the blood was allowed to drain directly into the centrifuge tube. The serum was separated from the clot by centrifugation. Microdeterminations were made for potassium by the method of Sobel and Kramer (1933) using trichloracetic acid filtrate; the value for the sodium was determined by the method of Weinbach (1935) using trichloracetic acid filtrate; and the value for chloride was determined by the method of Saifer and Kronblum (1935). Analyses for chloride were also made on 0.5 cc. of serum by the method of Wilson and Ball (1928) when possible. Only serum from unhemolyzed blood was employed in the determinations of potassium. It is recognized that ether anesthesia may produce small changes in the values for the serum potassium and possibly also in the values for sodium and chloride. For this reason, all conditions, including anesthesia, were kept as constant as possible. The values for the sugar were determined by the method of Folin (1929) by using blood which was aspirated from the hearts of animals which were deeply anesthetized with phenobarbital-sodium. Sodium oxalate was used as an anticoagulant. In our experience, phenobarbital-sodium may elevate the values for blood sugar of normal rats to a maximum of 10 per cent above the basal level. In the determinations of urea, blood was obtained from animals which were anesthetized either with ether or with phenobarbital-sodium.

The doses of cortin used in these experiments were selected on the basis of preliminary studies. The minimal amount for maintenance of the normal capacity for work under these experimental conditions was established. This amount was then doubled and this dosage was used throughout.

EXPERIMENTS AND RESULTS. In experiment 1, in order to obtain specimens of blood, nine normal rats were killed while they were under ether anesthesia and ten normal rats were killed while they were under phenobarbital-sodium anesthesia. The average values of the constituents of the blood are summarized in table 1.

In experiment 2, sixteen pairs of rats were subjected to both nephrectomy and adrenalectomy. Immediately after the operation and at each consecutive eight-hour interval thereafter, one rat of each pair was given a subcutaneous injection of cortin which was diluted to 2 cc., while the other

TABLE 1

Constituents of blood

	SODIUM		CHLORIDE		POTASSIUM		UREA		SUGAR	
1. Normal rats										
Number of rats	9		9		9		9		10	
Mean	140		102		5.1		52		101	
Coefficient of variation*	1		1		27		8			
2. Nonworking rats which had been subjected to adrenalectomy and nephrectomy										
	Cortin administered	No treatment	Cortin administered	No treatment	Cortin administered	No treatment	Cortin administered	No treatment	Cortin administered	No treatment
Number of rats	15	15	14	14	11	11	14	14		
Mean	140	140	102	104	10.5†	12.8†‡	356†	337†		
Coefficient of variation	8	6	7	4	12	13	18	23		
3. Working rats which had been subjected to adrenalectomy and nephrectomy										
Number of rats	13	13	13	13	10	10	11	11	13	13
Mean	144	146	101	105	6.4	8.4†‡	138†	130†	52†	37†‡
Coefficient of variation	5	7	15	8	26	23	29	9	26	25
4. Working adrenalectomized rats which received injections of water										
Number of rats	15	15	11	11	10	10	12	12		
Mean	120†	120†	91†	91†	8.2†	10.5†‡	81†	96†		
Coefficient of variation	5	7	4	8	8	19	31	40		

* Coefficient of variation and the reliability of the differences among means were calculated by formula given in standard textbooks on statistical methods.

† Reliably different from normal values.

‡ Reliably different from values for group which received cortin. In the summary of the data we have accepted those mean differences that had a t value 2.5 or greater as meeting statistical requirements for reliability.

animal of each pair was given an equal volume of distilled water. These animals were kept without additional anesthesia in the breeding cages with

a surplus of food and water. In every instance the rat which was receiving cortin was strong and active at the time of collapse of the untreated animal. The average time required for collapse to occur (omitting those animals which died unobserved) was thirty hours, with a range of eighteen to forty-eight hours. At the time of collapse, both animals of each pair were killed while they were under ether anesthesia and the blood was drained from the hearts. From 7 to 9 cc. of blood was consistently obtained from these animals and it was noted that the total volume of blood recovered by drainage was only slightly less in the untreated animal than it was in the animal which had received cortin. The average values for sodium, chloride, potassium and urea are summarized in table 1.

In experiment 3, twenty-seven pairs of rats were subjected to both adrenalectomy and nephrectomy. They were anesthetized with phenobarbital-sodium and were subjected to the continuous stimulation of the gastrocnemius muscle immediately after operation (4). One animal of

TABLE 2

Mean rates of work expressed as gram-centimeters per minute

	FOR ANIMALS WHICH HAD BEEN SUBJECTED TO ADRENALECTOMY AND NEPHRECTOMY		FOR ANIMALS WHICH RECEIVED INJECTIONS OF WATER	
	Cortin administered	No cortin administered	Cortin administered	No cortin administered
Number of animals	27	27	16	16
Initial rate	7024	6976	6600	6920
Initial range	4400 to 9600	4400 to 9600	4400 to 8000	4400 to 9600
Final rate	5560	800	5200	800
Final range	3200 to 7200	0 to 1200	3200 to 7600	0 to 1200

each pair received a subcutaneous injection of cortin immediately after operation and a second injection was administered at the six-hour period to animals which worked longer than six hours. The untreated animal of each pair received similar injections of distilled water. The animals were observed until the untreated one lost muscular responsiveness. The average time required for collapse of the animal which did not receive cortin was seven hours, with a range of four to ten hours. In every instance the animal which was receiving cortin still maintained a good rate of work at the time of collapse of the animal which did not receive cortin. The initial and the final rates of work are summarized in table 2. The data on the constituents of the blood are summarized in table 1. In this experiment the volume of blood which could be drained from the heart of the untreated animals at the time of collapse was only slightly less than that recovered from the heart of treated animals. The amounts recovered were between 4 and 6 cc.

In experiment 4, sixteen pairs of animals were adrenalectomized. Immediately after the operation 20 cc. of distilled water was injected intraperitoneally into each animal. They were anesthetized with phenobarbital-sodium and subjected to continuous stimulation of the gastrocnemius muscle. One animal of each group received a subcutaneous injection of cortin (diluted to a volume of 2 cc.) and the untreated animal received an injection of an equal volume of distilled water. The injection of cortin was repeated at intervals of six hours in those cases in which the animals worked longer than six hours. The experiment was continued until the untreated animals lost muscular responsiveness. The average time required for collapse to occur was five and a half hours, with a range of from three to nine and a half hours. In every instance the animal which was receiving cortin was still maintaining a good rate of work at the time of the collapse of the untreated animal. The initial and final rates of work are summarized in table 2. The data on the constituents of the blood are summarized in table 1. There was a striking difference in the volume of blood which could be recovered from the treated and untreated animals. In the untreated group the volume recovered varied from 2 to 3 cc., whereas the volume recovered from the treated animals varied between 4 cc. and 5.5 cc. The percentage of cells in the blood of untreated animals was significantly greater than the percentage in the blood of the treated animals.

Comment. In adrenal insufficiency the blood urea tends to increase above the normal value unless adequate amounts of cortin are administered. In the adrenalectomized rats which were also nephrectomized, treatment with cortin did not prevent an increase in the value for the blood urea equal to the values obtained for the untreated animals. It is evident that the animals treated with cortin could work at a normal rate, even in the presence of an unusually high concentration of blood urea.

The value for the blood sugar of the untreated adrenalectomized-nephrectomized rats which were subjected to work was always low at the time of their collapse. However, these values were not critically low and it is improbable that the collapse was caused by hypoglycemia. The values for the blood sugar of the animals which received cortin were also low and there was some overlapping of values between the two groups.

The influence of sodium chloride on the survival time of adrenalectomized rats has been shown by the addition of salt to the diet. We have shown that the administration of sodium chloride increased significantly the capacity for work in adrenalectomized rats. Whether or not sodium chloride exerts a favorable influence, depending on its concentration in the blood, is less evident, and that it does not primarily affect the state of insufficiency is indicated by the survival time and capacity for work of the adrenalectomized-nephrectomized rats. In the group of rats which had

been adrenalectomized and nephrectomized, the concentration of sodium and chloride in the serum was normal whether or not cortin was administered. Administration of cortin did not influence the distribution of sodium and chloride. In the adrenalectomized rats which received intraperitoneal injections of water, the concentration of sodium and chloride in the blood was much lower than normal. The injection of cortin did not increase the concentration of sodium and chloride in the blood serum of these rats but it did maintain their capacity for a normal rate of work. Swingle and co-workers (5) and Silvette and Britton (6) have observed that the severity of adrenal insufficiency does not depend on the values for sodium and chloride in the blood.

In contrast to this, the concentration of potassium in the blood serum was found to be greater than normal in all adrenalectomized-nephrectomized rats and in the rats which received intraperitoneal injections of water. The concentration of potassium was higher in the groups of rats that were not treated with cortin than it was in the rats that received cortin. It is therefore evident that cortin has an effect on the distribution of potassium between the tissues and the blood serum. In addition to its effect on the distribution of potassium, cortin maintained to a marked degree the capacity for work in both groups of adrenalectomized rats, those that had been nephrectomized and those that had received injections of water. These results indicate that cortin may be concerned with the permeability of the cells in regard to potassium and the effect of cortin on the capacity for work is associated with its favorable effect on the distribution of potassium. This effect of cortin is apparent, even in the presence of reduced concentrations of sodium and chloride. Swingle and co-workers (7) have shown that cortin has a beneficial effect on adrenalectomized dogs that have a low concentration of sodium and chloride in the blood.

It is important to note, however, that the concentration of potassium in the blood serum does not determine the state of adrenal insufficiency. The adrenalectomized-nephrectomized rats of the nonworking group which received cortin were strong and active and had a mean value for serum potassium of 10.5 m. eq. per liter, while the untreated adrenalectomized-nephrectomized animals of the nonworking group went into collapse with a mean value for serum potassium of 8.5 m. eq. per liter. It is well known that high values for the serum potassium are tolerated by some patients.

Similar observations in regard to the concentration of potassium in the blood and the severity of the symptoms of adrenal insufficiency in dogs have been reported (Allers, Nilson and Kendall, 8; Nilson, 9). The severity of the symptoms is related to the deficiency of cortin rather than to the concentration of potassium in the serum. The concentration of potassium, however, generally reflects the extent of cortical deficiency.

SUMMARY

In order to dissociate the influence of cortin on the tissues of the rat from its action on the kidney, the effect of cortin was determined on adrenalectomized rats after nephrectomy. Cortin did not prevent the increase in the amount of urea in the blood and did not change the concentrations of sodium or chloride which were within normal limits. It had a slight effect on the value for the blood sugar and a marked effect on the distribution of potassium. Cortin maintained a normal capacity for work which was quickly lost in the untreated animals. Loss of muscular activity was associated with an increase in the concentration of potassium in the serum.

The influence of cortin was determined on adrenalectomized rats that had received intraperitoneal injections of water. These rats, if untreated, had a low capacity for work, a low concentration of sodium and chloride and a high concentration of urea and potassium in the blood. Cortin maintained the capacity for work which was greatly suppressed in the untreated animals and retarded the rate of increase in the concentration of potassium in the serum but did not influence the concentration of urea, sodium and chloride.

Cortin exerts an effect on the tissues of the rat which is separate and distinct from its action on the kidney. This effect is apparently attributable to the influence of cortin on the permeability of cell membranes.

Sincere thanks are due to Miss E. R. Hartzler, M.S., for her aid in the chemical phases of these studies.

REFERENCES

(1) Loeb, R. F., D. W. Atchley and J. Stahl. J. A. M. A. **104:** 2149, 1935.
(2) Harrop, G. A., W. M. Nicholson and M. Strauss. J. Exper. Med. **64:** 233, 1936.
(3) Ingle, D. J. and E. C. Kendall. This Journal **117:** 200, 1936.
(4) Heron, W. T., W. M. Hales and D. J. Ingle. This Journal **110:** 357, 1934.
(5) Swingle, W. W., W. M. Parkins and A. R. Taylor. This Journal **116:** 430, 1936.
(6) Silvette, H. and S. W. Britton. This Journal **115:** 518, 1936.
(7) Swingle, W. W., W. M. Parkins, A. R. Taylor and H. W. Hays. This Journal **116:** 438, 1936.
(8) Allers, W. D., H. W. Nilson and E. C. Kendall. Proc. Staff Meet. Mayo Clinic **11:** 283, 1936.
(9) Nilson, H. W. This Journal. (In press.)

THE RELATION OF SOMATIC GROWTH TO THE MATURATION OF THE ERYTHROCYTE IN THE RAT[1]

ARTHUR H. SMITH AND GEORGE D. GROSS

From the Laboratory of Physiological Chemistry, Yale University, School of Medicine, New Haven

Received for publication September 15, 1936

The growth of the organism as a whole represents, under ordinary circumstances, a closely correlated development of various tissues, organs and body systems. However, accumulating evidence dealing with the chemical aspects of growth has shown that, depending on the nature of the nutritional adjustment, there occur alterations in the development of specific organ systems so that the usual correlation with the body as a whole is disturbed (see Smith, 1931). Thus independent changes in body weight, in body length, in size of organs, in size and composition of bone, in the composition of blood and other body fluids and in activity and learning have been noted in connection with specific alterations in the experimental ration.

Many studies on the blood of the albino rat have shown that from birth throughout the suckling period there is a slow increase in the number of erythrocytes with a more rapid rise, when solid food is consumed, to the adult level reached at about 105 days of age (Sure, Kik and Walker, 1929; Smith, 1932; Orten and Smith, 1934). Hemoglobin concentration, on the other hand, decreases immediately after birth and does not begin to rise above the birth level until after about 21 days of age (Jolly, 1909; Stucky and Brand, 1933). The adult level is reached at about 80 days of age in the rats of Sure, Kik and Walker and in those of Stucky and Brand, but at an age somewhat closer to 100 days in the animals described by Orten and Smith. The reticulocyte, regarded as an immature erythrocyte (Seyfarth, 1927; Sabin, 1928; Orten, 1933–34) constitutes from 80 to 99 per cent of the entire number of red cells in the blood of the albino rat at birth (Smith, 1932; Stucky and Brand, 1933; Orten and Smith, 1934); there is a rapid decrease until the adult level (less than 3 per cent) is reached at from 50 to 70 days of age. The present study records an attempt to determine whether restriction of growth of the body as a whole either by lack of food energy or by a specific amino acid deficiency, is accompanied by a retarda-

[1] The data discussed in this paper were taken from a thesis submitted by George D. Gross in candidacy for the degree of Doctor of Medicine, Yale University, 1936.

tion or other alteration of the development of the red cells as measured by the concentration of hemoglobin, the number of erythrocytes and the proportion of reticulocytes. In addition the effect of subsequent realimentation upon the foregoing criteria of hematogenesis was observed.

PROCEDURES. At weaning (21 days of age) male albino rats weighing 45 ± 5 grams were given an adequate ration (diet I, table 1) and 200 mgm. dried yeast daily; those animals reaching 110 ± 5 grams body weight in 15 ± 2 days were used for the experiment. They were then distributed to the experimental groups so that, in most cases, litter mates were placed in each of the three groups. The rats were housed in individual metal cages and body weight and food intake recorded. Group I continued to consume diet I ad libitum with 400 mgm. dried yeast daily for 14 weeks; group II

TABLE 1

Composition of experimental diets

	DIET I	DIET II	DIET III	
	per cent	*per cent*	*per cent*	
Lactalbumin*	18		36	The vitamin B complex was provided by feeding dried yeast‖ apart from the experimental ration. The amounts given are noted in the text
Gliadin†		18		
Dextrin‡	51	51	23	
Hydrogenated fat§	22	22	23	
Cod liver oil	5	5	10	
Salts¶	4	4	8	

* Crude, moisture 8.5 per cent, total nitrogen 12.0 per cent, ash 2.1 per cent, Dry Milk Company.

† Made from wheat gluten; moisture 3.0 per cent, total nitrogen 13.3 per cent.

‡ White, commercial.

§ Crisco.

¶ Osborne and Mendel (1917).

‖ Northwestern Yeast Company.

consumed ad libitum a ration (diet II) deficient in the essential amino acid lysine for 6 weeks and then were realimented with diet I for 8 weeks; group III consumed for 6 weeks weighed portions of diet III and then were realimented with diet I ad libitum for 8 weeks.

Diet II was adequate except that the only source of protein was gliadin which, on hydrolysis, yields too small an amount of the indispensable amino acid lysine to permit more than very slow increase in body weight under the experimental conditions employed. Diet III was qualitatively adequate but was restricted in amount given, so that the body weight of the rats in group III during the period of restriction, corresponded closely to that of the animals in group II. The concentrations of protein, cod liver oil and salts in diet III were elevated over those in the control ration (diet I) in order to eliminate the likelihood of a deficiency of any indis-

pensable factor other than energy when this ration was fed at the low level (ca. 2 gms. daily) required to restrict the weight of the animals of group III approximately to that of the rats in group II. When the control rats (group I) reached 300 grams body weight, the daily supplement of dried yeast was increased from 400 mgm. to 600 mgm. After realimentation the daily allowance of dried yeast for the animals of groups II and III was increased from 200 mgm. to 400 mgm.

Tail blood was used for the weekly hematologic determinations, care being taken to use only freely flowing blood and yet to prevent undue loss. The hemoglobin determinations were made with the acid hematin method using a glass standard calibrated by the oxygen capacity method and by iron determination. The erythrocyte counts were made in duplicate, using standard pipettes and counting chamber. The preparation of the reticulocyte slides and the enumeration of the cells followed closely the procedure described by Orten and Smith (1934).

Three to five rats were allocated to each group simultaneously. The entire experiment extending over a period of 14 weeks was repeated at three different times, viz., July to November, 1933; December to April, 1934; and August to January, 1935. Complete data were secured on 12 animals in each of 3 experimental groups.

Results. The experimental data have been presented in graphic form inasmuch as this method shows best the relationship of the changes in body weight to the shifts in hematologic values under the experimental conditions imposed. The data on individual rats show that the responses noted were not materially altered by seasonal changes; the data in the charts are, therefore, averages of values obtained on the entire 12 animals in each group.

Body weight. Growth of the rats in group I as exemplified by increase in body weight compared favorably with that described for normal stock animals by Smith and Bing (1928). In keeping with previous experience (see Osborne and Mendel, 1914), the rats on the lysine-deficient ration were strikingly retarded, gaining only about 3 grams per week over the 6-weeks' restriction period. During the same length of time the animals in group III increased in body weight slightly over 2 grams per week. This represents severe retardation in these rats weighing approximately 110 grams at the outset, as can be seen by the fact that during the same interval of time a typical animal of group I gained 28 grams per week.

Realimentation with unrestricted quantities of an adequate ration (diet I) produced in groups II and III prompt resumption of growth at a greatly accelerated rate. It is significant that, during the first two weeks after refeeding was begun, the average daily gain for the rats in group II was 5.5 grams and for those in group III, 7.0 grams. This rate of increase in body weight exceeds that of the control animals (group I) during the

period of most rapid growth and compares favorably with the extremely rapid growth demonstrated by Anderson and Smith (1932). Granting that a considerable portion of this gain in weight was water, there still is implied a very considerable strain upon those organ systems involved in the metabolism of the food materials consumed by the experimental animals.

Hemoglobin and erythrocytes. The increase in pigment and in red cells in group I was uniform and parallel from the beginning of the experimental period when the rats were 37 days old until the attainment of the adult level at about 110 days of age. This conforms with the observations previously cited.

In groups II and III the rate of increase and the values attained for hemoglobin during the period of restricted growth were much the same as

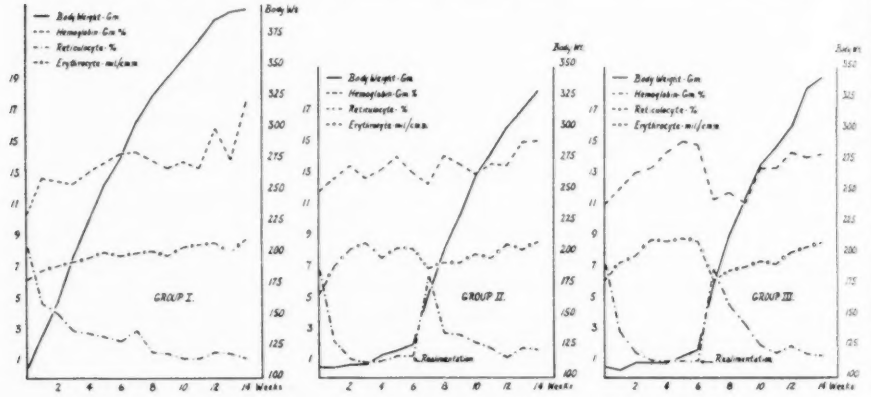


Chart 1

those observed in the normal control animals. However, the erythrocyte count reached the mature value somewhat more rapidly than did that of the control rats (group I). This observation is of particular interest in view of the comparative changes in proportion of reticulocytes to be discussed later. During the first week of realimentation, however, both groups, but particularly group III, showed a decline in concentration of pigment as well as of erythrocytes. During the first week of realimentation of group II there was a moderate decline to 12.5 grams hemoglobin but this value increased to 14.2 grams by the end of the second week, and reached a stationary level at 15.3 grams at the end of the eighth week. The erythrocytes of this group dropped from 8.2 millions to 7.1 millions during the first week of realimentation and slowly regained the adult level at the end of the sixth week. The drop in both pigment and red cell concentration

was more pronounced in group III; the hemoglobin decreased from 15.0 grams to 11.5 grams and the erythrocytes from 8.8 millions to 6.5 millions during the first week of realimentation and the return to normal values was observed only after 8 weeks. These somewhat more pronounced changes in the blood of this group may be correlated with the more rapid growth observed in these rats.

Reticulocytes. By the time the rats had been transferred to the experimental diet (37 days of age) the proportion of reticulocytes had already fallen to a level of about 8 per cent. The decrease continued in the control group (I) until, at approximately 5 weeks after the beginning of the experiment, the value was in the normal range.

In comparison with the control group (I), the animals in groups II and III showed, without exception, a more rapid decline in reticulocyte percentage so that the adult range was attained within 2 weeks after the beginning of the restriction in diet. It remained unchanged at this level throughout the remainder of the stunting period. Upon realimentation, the reticulocyte value in the blood of both groups showed a prompt and striking increase concomitant with the fall in hemoglobin and erythrocytes. The value in group II increased from 1.4 to 6.5 per cent; in group III the increase was from 1.1 to 7.0 per cent. In both groups the level of reticulocytes remained higher than that before realimentation for 5 to 6 weeks, after which the adult range was again observed.

DISCUSSION. It is obvious from the data obtained in the present study that the pronounced restriction of growth as measured by body weight is not accompanied by a similar retardation in the development of the blood as measured by the concentration of pigment, the change in number of erythrocytes, and the proportion of reticulocytes. Indeed, the blood seems to "mature" faster in the stunted animals than in the normal control rats, especially when the red cells and reticulocytes are the basis of comparison. There appears to have been demonstrated a dissociation between so-called somatic growth and the maturation of the red cells.

It is of interest to examine in greater detail, the responses of the hemopoietic mechanism to the metabolic demands brought about in the same animal by wide variations in the rate of change of body weight induced by nutritional adjustments. In the normally growing rat the steadily-increasing demands for gas exchange is accommodated by a regularly augmented concentration of erythrocytes and total volume of blood until at about 105 days of age, the mature composition is reached. This steady demand for erythrocytes is satisfied so efficiently that already at 50 to 70 days of age the proportion of reticulocytes—the index of accelerated red cell formation—has reached an adult level. In the rats whose body mass is early prevented from enlarging, as in groups II and III in the present experiment, the total erythrocyte demand (concentration x blood volume)

is suddenly stabilized. Hemopoietic inertia, at this stage of ordinarily rapid maturation, brings about a somewhat more rapid rise in red cell concentration to the adult level and, at the same time, due to diminished demand for new erythrocytes, the proportion of reticulocytes falls unusually rapidly. This view is supported by the observation of Fisch and Emmel (1924) that polychromasia and reticulosis in the blood of rats disappear during inanition.

Upon realimentation with unrestricted amounts of an adequate ration, resumption of growth is prompt and the rate of increase in body weight is higher than usual either for age or for size. New tissue is rapidly formed, the capillary bed expands and the demand for fluid to fill the blood vessels exceeds the capacity of the blood-forming tissue to provide red cells. The result is a prompt drop in concentration of erythrocytes and pigment in the circulating blood essentially through dilution. That the hemopoietic mechanism responds vigorously to the requirement for new cells is shown by the concomitant rise in proportion of reticulocytes to a level considerably higher than the normal range. The data further indicate that during the period of very rapid increase in body weight, although the erythrocyte concentration returns rapidly to the adult level, the blood-forming mechanism is under considerable stress as shown by the slow fall in the proportion of reticulocytes. It is also apparent that pigment formation is favored, during this period, by the unrestricted consumption of the complete ration.

The foregoing interpretation of the observations described herein would seem to be supported by the fact that both the methods employed for retarding growth are known to produce comparatively mild changes in the organism. Furthermore, these two devices are dissimilar, from the nutritional point of view, yet produce essentially the same disturbance in the blood picture. Again, a similar change in the number of red cells, the concentration of hemoglobin and proportion of reticulocytes has been observed when realimentation consisted in the addition of inorganic salts to a mineral-poor experimental diet.[2, 3]

The foregoing observations provide further evidence that the correlative feature of growth can be eliminated to a greater or less extent by suitable nutritional devices. The organs and systems of the body are linked by functional integration, a factor to which so-called differential rates of growth is due, at least, in part. A more clean-cut approach to this question can be made when the gain in body weight can be more clearly defined through a more precise knowledge of the chemical nature of the tissue elaborated by the animal at different stages of its development.

[2] Unpublished observations in this laboratory.

[3] Rosenstern (1911) states that during realimentation after inanition there occurs a more rapid regeneration of plasma than of the other components of the blood.

SUMMARY

Restriction of somatic growth, as measured by changes in body weight and produced either by deficiency of food energy or of the essential amino acid lysine, does not prevent the usual progress of changes in the concentration of pigment, number of erythrocytes and proportion of reticulocytes in the blood from infancy to early adulthood in the albino rat.

The generally-observed inverse relationship between the number of erythrocytes and the concentration of hemoglobin on the one hand and the proportion of reticulocytes, on the other, exists both in normal animals and in those stunted by either of the nutritional deficiencies employed and is greatly emphasized during the period of accelerated growth following realimentation.

The mature blood picture is attained earlier in the restricted rats than in those permitted to grow on an adequate ration; realimentation is followed by changes in body weight, number of erythrocytes, concentration of pigment and proportion of reticulocytes which are explained on the basis of functional response.

REFERENCES

ANDERSON, W. E. AND A. H. SMITH. This Journal **100:** 511, 1932.
FISCH, M. E. AND V. E. EMMEL. Anat. Rec. **27:** 181, 1924.
JOLLY, J. Compt. rend. Soc. Biol. **66:** 136, 1909.
ORTEN, J. M. Yale J. Biol. and Med. **6:** 519, 1933–34.
ORTEN, J. M. AND A. H. SMITH. This Journal **108:** 66, 1934.
OSBORNE, T. B. AND L. B. MENDEL. J. Biol. Chem. **17:** 325, 1914.
J. Biol. Chem. **32:** 309, 1917.
ROSENSTERN, I. Ergeb. d. inn. Med u. Kinderheil., **7:** 332, 1911.
SABIN, F. Physiol. Rev. **8:** 191, 1928.
SEYFARTH, C. Folia haematol. **34:** 7, 1927.
SMITH, A. H. J. Nutrition **4:** 427, 1931.
SMITH, A. H. AND F. C. BING. J. Nutrition **1:** 179, 1928.
SMITH, C. J. Path. and Bact. **35:** 717, 1932.
STUCKY, C. J. AND E. BRAND. Proc. Soc. Exper. Biol. and Med. **30:** 932, 1933.
SURE, B., M. C. KIK AND D. J. WALKER. J. Nutrition **1:** 299, 1929.

A STUDY OF THE AUGMENTATION OF OVARIAN WEIGHTS AS EFFECTED BY ZINC SULPHATE, ANTUITRIN S AND THYROID IMPLANTS

FREDERICK E. EMERY

From the Department of Physiology, University of Buffalo

Received for publication September 15, 1936

The explanation of the activation of pituitary extracts by the urine hormone, prolan, has undergone considerable change since it was first discussed by Evans *et al.* (1). This synergistic effect of prolan and special prepared synergists (2) (3) may be due, according to the recent report of Saunders and Cole (4), to delayed absorption of the active principle. This would also account for the synergistic effects obtained with $ZnSO_4$ (5) and protein substances (4).

In cases where pituitary implants are given, the absorption of the gonad-stimulating hormone continues for a period of 8 days or more (6). In these animals one would not expect $ZnSO_4$ to have any synergistic effect if the mechanism was due to slow absorption, because absorption is already extended beyond the period necessary to bring about marked increases in ovarian weight. Experiments to support this hypothesis, together with data on the synergistic effect of $ZnSO_4$, thyroid tissue and Antuitrin S, are herein discussed.

Immature rats 25 days of age were used. Antuitrin S was given intraperitoneally in 2 injections, one at the start and the second, the next day; the 2 injections totaled 50 rat units. The pituitary implants were made either intraperitoneally or intramuscularly. A one-tenth per cent $ZnSO_4$ solution was given in doses of 2 mgm., to a total of 10 mgm. in 4 days. The $ZnSO_4$ was given either intraperitoneally or subcutaneously as indicated. All rats were killed 5 days after the beginning of the experiment and the ovaries, uterus, and adrenal glands carefully dissected free of other tissue and weighed. Since the uterus confirms the ovarian changes and the adrenals add little to the present discussion, these data are omitted from table 1.

RESULTS. *The effect of $ZnSO_4$ combined with Antuitrin S.* The results obtained with 50 R.U. of Antuitrin S are shown in group B, table 1. The ovaries were increased in weight 79 per cent and the mean weight of 22.1 mgm. is a significant gain over the controls (group A). These data are in accord with the expected results from treatment with Antuitrin S. With

identical procedure in respect to Antuitrin S but with 10 mgm. of $ZnSO_4$ given intraperitoneally, the ovaries weighed 28.4 mgm. and were 135 per cent heavier than the controls (group C). The mean difference in ovarian weight between this group and the previous one though small is nevertheless significant, the mean difference divided by the standard error of the difference being 3.2, as shown in the last column of the table. Quite in contrast to this are the results obtained when the same amount of $ZnSO_4$ solution was given subcutaneously. The ovaries in these rats (group D) were not

TABLE 1

The treatment and ovarian weights in milligrams for the several groups of rats are shown

Autopsy at 30 days of age. The standard error of the difference was calculated by the formula

$$\sqrt{(S.E.^1)^2 + (S.E.^2)^2}$$

where S.E.^{1} and S.E.^{2} are standard errors of the two means that are being compared. IP = Intraperitoneally. SQ = Subcutaneously. Antuitrin S given in rat units. M = Implants in the muscles.

GROUPS	NUMBER OF RATS	ANTUITRIN S	PITUITARY IMPLANTS	$ZnSO_4$	MEDIAN	MEAN	PER CENT INCREASE	GROUPS COMPARED	MEAN DIFFERENCE ÷ S.E. OF DIFFERENCE
A	12	None	None	None	13.0	12.9			
B	15	50 R.U. IP	None	None	20.0	22.1	79.1	A, B	5.6
C	16	50 R.U. IP	None	10 mgm. IP	28.0	28.4	135.6	B, C	3.2
D	9	50 R.U. IP	None	10 mgm. SQ	22.0	20.9	54.3	B, D	−1.2
E	14	None	1 ♂ IP	10 mgm. IP	15.0	16.4	27.1	E, K	0.4
F	10	50 R.U. IP	1 ♂ IP	None	60.5	59.8	372.0	B, F	12.3
G	9	100 R.U. IP	None	None	25.0	26.8	115.5	B, G	1.8
H	12	None	1 ♂ IP	None	15.0	14.6	13.2	A, H	1.4
I	10	None	2 ♂ IP	None	51.0	50.3	290.0	H, I	9.2
J	12	None	*	None	14.0	15.2	17.8	J, K	0.1
K	30	None	1 ♂ M	None	15.0	15.4	19.5	H, K	0.4

* One normal ♂ and two thyroids in muscles.

significantly different than in those (group B) where the $ZnSO_4$ was omitted, the means being 20.9 and 22.1, respectively.

Pituitary implants not affected by $ZnSO_4$. In order to make the implant experiments similar to those described in the previous section the implants were made intraperitoneally. This procedure gave an opportunity to place both pituitary tissue and $ZnSO_4$ in a common cavity. One normal male pituitary gland gave a minimal response. The ovaries weighed 14.6 mgm. (group H) and were only 13 per cent heavier than the controls. The vaginae were opened and gave positive smears in all these rats. The posi-

tive vaginal reactions with minimal effects on ovarian weight were the same as we previously pointed out for intramuscular implants of one male rat pituitary gland (7). In fact there is no significant difference between intraperitoneal and intramuscular implants (compare groups H and K). When $ZnSO_4$ was given intraperitoneally in addition to one male pituitary gland the effect on the ovaries was the same as found when the $ZnSO_4$ was not given (group E). A comparison of groups E and K leads one to conclude that $ZnSO_4$ does not change the reaction of a minimal pituitary implant stimulus.

The effect of doubling the dose of Antuitrin S and pituitary implants. A few years ago considerable interest centered around the fact that the urine hormones of pregnancy increased the weight of the ovaries to some extent and beyond this, increasing the dosage had no effect. In contrast to this the pituitary hormones on the other hand readily summate (8). In order to see more clearly the synergistic effect of Antuitrin S and pituitary implants, let us first see what happens when a double dose (100 R.U.) of Antuitrin S is given. The ovaries were somewhat larger than when 50 R.U. was given, the mean weights being 26.8 and 22.1 mgm. respectively. The mean difference is small and not significant (group G). Quite in contrast are the results obtained by doubling the amount of implanted pituitary tissue. The mean ovarian weights increased from 14.6 to 50.3 mgm. (groups H and I). The mean difference divided by the standard error of the difference was 9.2, as indicated in table 1.

With these results in mind the striking synergistic effect of Antuitrin S and pituitary implants became evident. Thus, when 50 R.U. of Antuitrin S was given in combination with one male rat pituitary, the ovaries weighed 59.8 mgm. (group F). Although others have reported similar results with urine synergists and pituitary extract (9), (10), it seems that the synergistic effect of Antuitrin S and pituitary implants has not previously been reported.

Pituitary implants in combination with thyroid implants. The augmentation of ovarian weight that Saunders and Cole (4) obtained with their pituitary synergist plus egg albumin or casein gave reason to expect that a similar result might be obtained with other proteins and pituitary implants. To test this the entire thyroid gland from an adult male rat was implanted with the pituitary gland in the muscles of immature rats. Neither the proteins nor the hormones from the implanted thyroid tissue had any effect in these experiments, and the results were not different from those obtained by the pituitary implant alone (compare groups J and K).

Discussion. The augmentation of Antuitrin S by $ZnSO_4$ is interesting in the light of Maxwell's (5) report that he failed to obtain it. This augmentation, though small, is consistent and, as previously mentioned, the change in the weight of the ovaries is significant. Since the Antuitrin S

was given in 2 injections, one at the start of the experiment and the second the next day, it is not unlikely that the $ZnSO_4$ delayed absorption. Yet it is apparently not clear that delayed absorption could explain these results. Evans and Simpson (11) failed to get larger ovaries by dividing the dose of prolan and giving it at short intervals.

Very striking effects on the ovarian weight were obtained with Antuitrin S and pituitary implants (group F). The question as to how this is brought about cannot at present be answered. Evans *et al.* (10) thought that the urine hormone activated a prohormone of the hypophysis. More recently the cause has been thought to be due to delayed absorption as Maxwell (5) has shown for combinations of pituitary extract and $ZnSO_4$ and Bischoff (12) for insulin and zinc. Saunders and Cole (4) likewise explained the augmentation of $ZnSO_4$ in this way and state further that the same applies to proteins. It seems that the $ZnSO_4$ must be concentrated at the locus of absorption and has no effect if administered in adjacent tissues as was shown when Antuitrin S was injected intraperitoneally and $ZnSO_4$ subcutaneously (group D).

The lack of any augmentation when $ZnSO_4$ and pituitary implants were both given intraperitoneally is not easy to evaluate. The suggestion offered is that absorption is going on so slowly that the zinc does not appreciably change it. At least there is evidence for slow absorption in the fact that pituitary implants become vascular and continue to supply the sex hormone for some time (6). But until a clearer conception of the basis underlying the synergistic augmentation of ovarian weight is presented, discussion seems futile.

SUMMARY

Several groups of immature rats have been used to determine the effect of $ZnSO_4$ in the augmentation of ovarian weights. In combination with Antuitrin S and given intraperitoneally, the ovaries were significantly larger than in similar rats without the $ZnSO_4$. The $ZnSO_4$ had no effect when injected subcutaneously and the Antuitrin S intraperitoneally.

Some increase in ovarian weight was obtained by doubling the dose of Antuitrin S, much more by doubling the amount of implanted pituitary tissue, and the greatest effect of all when Antuitrin S was given with pituitary implants.

Neither proteins as represented by thyroid tissue nor $ZnSO_4$ had any effect on the results obtained with pituitary grafts.

REFERENCES

(1) Evans, Meyer and Simpson. Proc. Soc. Exper. Biol. and Med. **28:** 845, 1931.
(2) Evans, Simpson and Austin. J. Exper. Med. **58:** 545, 1933.
(3) Saunders and Cole. Proc. Soc. Exper. Biol. and Med. **33:** 504, 1936.
(4) Saunders and Cole. Proc. Soc. Exper. Biol. and Med. **33:** 505, 1936.

(5) Maxwell. This Journal **110:** 458, 1935.
(6) Emery. Anat. Rec. **66:** 253, 1936.
(7) Emery, Bash and Lewis. Proc. Soc. Exper. Biol. and Med. **29:** 42, 1931.
(8) Swezy. J. Lab. and Clin. Med. **19:** 561, 1934.
(9) Leonard. Proc. Soc. Exper. Biol. and Med. **30:** 403, 1933.
(10) Evans, Meyer and Simpson. This Journal **100:** 141, 1932.
(11) Evans and Simpson. This Journal **89:** 381, 1929.
(12) Bischoff. This Journal **116:** 239, 1936.

PANCREATECTOMY IN THE PIG

F. D. W. LUKENS

From the George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia

Received for publication September 17, 1936

The majority of the work on pancreatectomy has been done in the dog or cat. The few studies that have been made in other species indicate that pancreatic diabetes may present a somewhat different picture than that seen in the carnivora. The recent demonstrations that diabetes is modified by hypophysectomy or adrenalectomy and the availability of potent pituitary extracts suggested another approach to the study of the species differences in pancreatic diabetes. These alterations in diabetes have been produced chiefly in the dog and cat. The pig offers a contrast to the carnivora. It is an ungulate, is omnivorous, and has a tendency to become obese. A series of young pigs has therefore been depancreatized, and the results reveal certain differences from the dog and cat which will be described.

In 1893 Minkowski (1) reported the results of partial pancreatectomy in one pig. He noted that the operation did not produce the severe diabetes typical of other mammals. In 1911 Gould and Carlson (2) depancreatized two pigs. They likewise stated that the resulting diabetes was mild, but no data on glucose excretion were given at that time. Later, Carlson and Drennan (3) performed complete pancreatectomy on two pigs and reported some of the metabolic results. One pig died in 48 hours—too short a period for satisfactory studies. The other was fed and lived 66 days, although no pancreas was found at autopsy. The fasting metabolism was not fully studied. No further mention of pancreatectomy in the pig has been found, although the blood sugar curves and associated electrolyte changes in normal pigs have been described by Eveleth (4) (5).

Methods. Young pigs of both sexes weighing 5 to 15 kgm. have been used. Prior to operation they were fed unlimited amounts of midlings (raw corn meal) with small amounts of raw fruit and vegetables and vitamine preparations. Pancreatectomy is difficult in the pig because of the intimate relation of the pancreas to the portal vein. The operation was successful in 9 of 12 pigs in which it was attempted. Nembutal anesthesia has been used throughout. The pig has a small margin of safety so that the required dose of 40 to 45 mgm. per kilogram intraperitoneally should

be given cautiously. The few slight wound infections were considered negligible. Following operation the animals were kept in metabolism cages. Feeding, urinalyses, etc., will be described in the results.

Blood sugar was determined by Benedict's method, urine glucose by the Benedict method, urine nitrogen by the micro Kjeldahl, urine acetone bodies by the method of Van Slyke, liver and serum fatty acid by a modification of the Stoddard-Drury method.

RESULTS. The consequences of pancreatectomy in the pig may be described under the following headings:

Survival. The pigs survived for from 8 to 15 days. Feeding did not prolong the lives of these animals. The survival period of these pigs is comparable to that of the dog, but is quite at variance with the depancreatized pig reported by Carlson and Drennan (3) to have lived 66 days.

Weight loss. This is marked in the depancreatized pig and is not prevented by food. However, for a given period of time, their rate of loss is roughly comparable to the fasted animal. This is unlike the cat or dog in which pancreatectomy causes a much more rapid loss of weight than occurs in fasted controls. The absence of glycosuria to be referred to below may be related to this.

Behavior and mode of death. The pigs have been lively and well, except for slight weakness, until death. They appear to die from inanition, exhaustion and, in at least a few instances, from hypoglycemia. In spite of marked acetonuria they did not develop coma, and as subsequent blood studies will show, they displayed no severe acidosis. The failure to develop severe acidosis in the presence of ketosis is unlike the cat or dog. The induction of acidosis and coma by pituitary extracts will be noted later.

Autopsy. In all the animals reported no pancreas has been found at autopsy which included microscopic section of all doubtful tissues. In addition the autopsy findings revealed a loss of the fat depots and fatty changes in the liver and kidneys.

Glucose, nitrogen and acetone body excretion during fasting. When the animals were fasted, the glycosuria was very slight or absent, ranging from 0 to 0.5 gram per kilo per day with an average of 0.24. This is quite different from the dog and cat. It is, however, comparable to cats which have had the pituitary or adrenals removed as well as the pancreas (6). These doubly operated animals are frequently sugar free when fasted.

The nitrogen excretion of the depancreatized pig is lower than that of the cat, the average being 0.5 gram per kilo per day for the pig (range 0.4–0.5) and 1.4 for the cat.

Four fasted control pigs had an average nitrogen excretion of 0.26 gram per kilo per day. It is apparent that although the nitrogen excretion in the diabetic pig is lower than in the case of the diabetic cat, it has, never-

theless, been doubled by pancreatectomy. The D/N ratio is low and variable as in the case of the hypophysectomized diabetic cat.

The average figures for acetone body excretion fail to portray the intensity of the ketonuria that develops in these animals. Acetone bodies appear about the second day, and then rapidly rise until as much as 4000 mgm. a day are excreted. This is illustrated in the protocol of pig 11, table 1.

Glucose tolerance tests. A starvation glycosuria of normal pigs has been found that is in agreement with the findings of Carlson and Drennan (3). In contrast, three oral sugar tolerance tests on two fasted depancreatized

TABLE 1

Protocol of pig 11

DATE	URINE				REMARKS
	Volume	Glucose	Nitrogen	Acetone bodies	
	cc. per day	*grams per day*	*grams per day*	*grams per day*	
2-27 Pancreatectomy					Weight 8.0 kilos
2-28	245	0	4.4	0.00	
2-29	166	0	3.1	0.11	
3-1	255	6.6	1.9	0.25	10 grams glucose orally
3-2	230	0	2.0	1.23	400 grams lean beef
3-3	435	5.8	7.4	1.98	170 grams lean beef
3-4	450	2.7	7.7	3.00	355 grams lean beef
3-5	510	27.3	4.6	3.09	20 grams sucrose orally
3-6	520	20.8	3.5	1.60	20 grams sucrose orally
3-7	220	0	2.2	3.50	
3-8	260	0	2.4	4.59	
3-9	220	0	2.3	2.56	
3-10	270	0	2.7	3.73	
3-11	Nembutal and autopsy				Weight 4.7 kilos

pigs reveal quantitative excretion of ingested sucrose. This is illustrated in table 1 and conforms to the behavior of the diabetic dog. It is even more like the behavior of the adrenalectomized or hypophysectomized diabetic cat, in which the complete excretion of ingested carbohydrate may occur with no glycosuria before or after the test period. It would appear that the "mild" diabetes of the pig is not due to the ability to utilize glucose in appreciable amounts in the absence of insulin.

Terminal serum chemistry. Table 2 summarizes the terminal serum chemistry of pigs dying of pancreatic diabetes with that of two normal pigs for comparison. The blood sugar values are low or only moderately elevated. The urea N. is definitely elevated as it is in the depancreatized

cat. The CO_2 combining power is remarkably little affected. This is unlike the cat and dog and is all the more unexpected in view of the marked acetone body excretion. Total base is relatively well maintained although the chlorides are consistently reduced. An unusual ability to excrete ammonia might account for this, but we have no data on ammonia. Cholesterol, fatty acids and lipoid phosphorus are increased, but only to a slight degree.

TABLE 2

Terminal serum chemistry

PIG NUMBER	DAYS AFTER PANCREATECTOMY	PITUITARY EXTRACTS	FATTY ACIDS	CHOLESTEROL	LIPOID PHOSPHORUS	CO_2 COMBINING POWER	SUGAR	UREA NITROGEN	CHLORIDES	TOTAL BASE
Normal										
		cc.	*grams per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*vols. per cent*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*m.eq./l.*	*m.eq./l.*
11			0.21	85	5	51	110	13	98	142
18			0.25	104	7	56	86	12	99	144
Depancreatized—no extract										
5	5	0	0.24	159	6	45	160	57	66	
10	7	0	0.27	156	6	72	232	23	92	139
11	13	0	0.32	139	7	53	62	20	87	137
16	15	0	0.34	108	6	64	246	27	82	132
Average.....			0.27	140	6	58	175	32	82	136
Depancreatized—extract										
9	13	5*	1.64	183	16	19	176	37	83	146
14	5	17†	4.87	375	27	13	60	37	65	120
6	4	20†	4.05	439	31	25	460	85	87	141
Average.....			3.52	332	25	19	232	53	78	136

* Survived 1 day after injection of pituitary extract.

† Survived 2 days after injection of pituitary extract.

The analyses in this table have been carried out by Dr. F. W. Sunderman of this department.

Effect of pituitary extracts. Three pigs (6, 9, 14) were injected hypodermically or intraperitoneally with pituitary extracts. The preparations used were crude alkaline extracts of carefully dissected anterior lobes (beef). All these animals developed the clinical picture of acidosis and coma and died one or two days after the extract was given. Glucose excretion was increased by the extract although the amount of this increment varied widely. Thus in pig 6 the glucose rose from 10 to 26 grams,

in pig 9 from 12 to 17 grams and in pig 14, which had been sugar free, 2.4 grams of glucose were excreted on the day extract was given. In pig 6 the nitrogen excretion was greatly increased; in 9 and 14 there were small increases in the nitrogen which were within the experimental limits. Ketonuria was increased but in amounts within the natural variation of these animals. The most striking change in the extract treated animals, however, was that noted in their terminal serum chemistry (table 2). The CO_2 combining power is that of severe acidosis. The serum fatty acids are increased as much as 10 or 15 times, cholesterol and lipoid phosphorus 4 to 5 times. Such an extensive alteration of these substances far exceeds the changes reported by Mathews, Newton and Bloor (7) for depancreatized cats. Except for the degree of the increase in serum lipoids the response of the depancreatized pig to pituitary extracts resembles in all its characteristics the response of the hypophysectomized-depancreatized cat to similar extracts (11).

Liver fatty acids. The livers of all nine animals were grossly fatty, and were analyzed for fatty acids. The values ranged from 4.2 to 28.1 grams per cent with an average of 13.6. No determinations have been made on normal pigs. No distinct difference was observed in those pigs receiving pituitary extracts. This is noted in view of the marked rise in blood fats described above. The wide range of liver fats renders conclusions impossible in such a small series. They seem to conform to the findings in the depancreatized dog or cat.

Discussion. Pancreatectomy in the pig is followed by a low glucose excretion, a low although increased nitrogen excretion, marked ketonuria without severe acidosis, the quantitative excretion of ingested sucrose and a low and variable D/N ratio. The features in which these results differ from those of the depancreatized carnivorous animal have been noted.

Depancreatized pigs manifest a close similarity to cats in which the pituitary or adrenals have been removed as well as the pancreas; in fact they seem to fulfill the whole picture of the hypophysectomized-depancreatized carnivor except for the presence of ketonuria. Even with this ketonuria they behave like the doubly operated cat in not developing acidosis and coma. In the development of these symptoms after the injection of anterior pituitary extracts the pig is quite like the hypophysectomized diabetic animal.

A comparison of the depancreatized pig with other types of depancreatized animals is presented in table 3 which summarizes the chief points of similarity and difference that have been mentioned. The pig has $\frac{1}{10}$ to $\frac{1}{15}$ the glucose excretion and $\frac{1}{2}$ to $\frac{1}{3}$ the nitrogen excretion of the carnivor. The pig's glucose and nitrogen excretion is comparable to that of the doubly operated cats from which the pig differs in having a marked ketonuria. The remarkable survival and blood sugar studies reported by Sprague and

Ivy (9) in the duck represent the most striking species difference in the effect of pancreatectomy. It is unfortunate that the duck is not suitable for metabolic experiments. Preliminary studies on the depancreatized goat indicate that its diabetes may be even milder than that of the pig. From table 3 it is evident, as stated by Long and Lukens (10) that pancreatic diabetes may be modified by the selection of different species of animals or by the simultaneous removal of other endocrine organs in a single species. Further work with such methods should enlarge our conception of the diabetic syndrome.

A few suggestions may be made concerning the marked ketonuria with little or no glycosuria. As Lusk has said, the pig is the "supreme converter

TABLE 3

Comparative effects of pancreatectomy in different species

ANIMALS	NUMBER OF ANIMALS	SURVIVAL	URINE			D/N	BLOOD SUGAR
			Glucose	Nitrogen	Acetone		
A. Species differences							
		days	*g./k./d.**	*g./k./d.**	*mgm./k./d.**		*mgm. per cent*
Dog (Houssay, 8)	4	10	2.8	1.0		2.8	310–345
Cat (Long and Lukens, 6)	10	5	3.2	1.4	133	2.8	212–788
Pig	5	9	0.2	0.5	179	0.4	30–232
Duck (Sprague and Ivy, 9)	12	110					100–200
B. Operative procedures in one species							
Cat (hypophysectomized-depancreatized, 6)	6	49	0.7	0.7	8	1.0	40–320
Cat (adrenalectomized-depancreatized, 6)	14	17	0.5	0.7	10	0.7	30–308

* Grams or milligrams per kilo per day.

of maize to animal fat." The stimulus to this conversion is presumably a high blood sugar. When the blood sugar is raised by pancreatectomy glucose may be converted to fat with such facility that it escapes excretion. In the absence of normal carbohydrate utilization fat is burned in excessive quantity giving rise to the ketonuria. Since this ketonuria fails to cause acidosis the pig must have an unusually effective mechanism for eliminating organic acids. The preservation of the total base of the blood in spite of a loss of chloride suggests that this is the explanation. Ammonia formation by the kidney may be more efficient in the pig than in other animals.

Carlson and Drennan (3), referring to the mild character of diabetes in

the pig, suggested that this species might have an "inherent ability of the tissues to use carbohydrate." *a.* By the methods here employed there is no appreciable utilization of carbohydrate. *b.* By analogy there is great similarity between the depancreatized pig and the hypophysectomized-depancreatized carnivor. *c.* By pituitary extracts the pig is made more severly diabetic. Therefore, it is suggested that the pig has some basic difference from the carnivor in the kind or degree of its pituitary function, and that the functional state of the pituitary (or some other endocrine organ activated thereby) accounts for the mild appearance of porcine diabetes.

SUMMARY

Nine young pigs have been depancreatized, the average survival being nine days. Although ingested carbohydrate was quantitatively excreted, the fasting glucose excretion was slight or absent, and the fasting nitrogen excretion was low. Ketonuria was marked but was not accompanied by severe acidosis. The injection of pituitary extracts produced acidosis, coma and greatly increased serum lipoids.

The results have been compared with pancreatic diabetes in other species and with the findings in hypophysectomized or adrenalectomized diabetic animals. It is suggested that the seemingly mild diabetes of the pig is due to the nature of its pituitary (or other endocrine) function rather than to the utilization of carbohydrate in the absence of insulin.

REFERENCES

(1) Minkowski, O. Arch. f. exper. Path. u. Pharmakol. **31:** 83, 1893.
(2) Gould, L. K. and A. J. Carlson. This Journal **29:** 165, 1911.
(3) Carlson, A. J. and F. M. Drennan. J. Biol. Chem. **13:** 465, 1912.
(4) Eveleth, D. F. J. Biol. Chem. **104:** 559, 1934.
(5) Eveleth, D. F. and F. M. Eveleth. J. Biol. Chem. **111:** 753, 1935.
(6) Long, C. N. H. and F. D. W. Lukens. J. Exper. Med. **63:** 465, 1936.
(7) Mathews, V. J., J. K. Newton and W. R. Bloor. J. Biol. Chem. **108:** 145, 1935.
(8) Houssay, B. A. and A. Biasotti. Endocrinol. **15:** 511, 1931.
(9) Sprague, R. and A. C. Ivy. This Journal **115:** 389, 1936.
(10) Long, C. N. H. and F. D. W. Lukens. Tr. Assoc. Am. Physicians. (In press).
(11) Long, C. N. H. and F. D. W. Lukens. This Journal **116:** 96, 1936.

COMPARATIVE INFLUENCE OF EPINEPHRINE AND OF DEXTROSE ON THE UTILIZATION OF SUGAR BY THE MUSCLES, DETERMINED WITH THE AID OF THERMOSTROMUHR MEASUREMENTS OF BLOOD FLOW

S. SOSKIN, HIRAM E. ESSEX, J. F. HERRICK AND FRANK C. MANN

From The Metabolic Laboratory, Department of Physiology, Michael Reese Hospital, Chicago, Illinois, and the Division of Experimental Medicine, The Mayo Foundation, Rochester, Minnesota

Received for publication September 19, 1936

In a communication by Soskin, Priest and Schutz, the available evidence for the hypothesis that epinephrine hyperglycemia results chiefly from decreased utilization[1] of sugar by the muscles was summarized and critically considered. Evidence was then presented to show that the arteriovenous blood sugar difference on which the foregoing idea was largely based, cannot be used as a quantitative estimate of utilization of sugar by the muscles, unless it is corrected for rate of blood flow and hydration of the blood, and determined at frequent intervals. When the arteriovenous blood sugar differences, obtained under the appropriate experimental conditions, were so corrected, no support could be found for the belief that epinephrine decreases the utilization of sugar by the muscles. A simple glass and rubber flowmeter of original design was used in the work cited.

In view of the foregoing findings, Cori, Fisher and Cori used the thermoelectric device described by Gibbs as an index of the rate of blood flow, in subsequent experiments in which they observed the influence of epinephrine and of insulin on the arteriovenous blood sugar difference. Since this device did not enable them to measure actual blood flow, they scaled down their dosages of epinephrine until the amounts used caused no apparent change in the rate of flow (within 10 to 15 per cent). They found that these smaller dosages of epinephrine (a fourth to an eighth of those previously used in work with dogs) changed the arteriovenous blood sugar difference from an average of 2 mgm. per cent before injection to 3 to 4 mgm. per cent during the injection. This small increase in arteriovenous difference they contrasted with differences of 10 to 25 mgm. per cent caused by the administration of insulin, the dosage of which was not recorded.

[1] In the present communication, the terms "utilization" or "retention" are used to indicate the sum total of the processes which operate in the disposal of sugar within the muscle, while "oxidation" is strictly reserved for that degradation process by which carbon dioxide and water are formed.

The results of Cori, Fisher and Cori, thus briefly summarized, doubtless indicate that small amounts of epinephrine may cause less increase in the utilization of sugar by the muscles than do certain unstated amounts of insulin. They do not, however, show any decrease in utilization of sugar as a result of administration of epinephrine. Nor did the investigators present any further data comparing utilization of sugar during comparable levels of hyperglycemia, caused by administration of epinephrine and of dextrose respectively, a comparison on which their original thesis was based. Nevertheless, these authors reaffirmed their belief that epinephrine decreases utilization of sugar and they pointed out two objections to the opposing evidence.

The criticisms which Cori, Fisher and Cori made of the work of Soskin, Priest and Schutz were as follows: 1. The solutions of epinephrine used were not protected with anti-oxidants and hence the effective dosages were less than those administered. 2. The blood flow gradually declined to extremely low values in each experiment.

The solutions of epinephrine undoubtedly lost some of their potency. These solutions were based on the pertinent previous papers of Cori, Fisher and Cori and of Colwell and Bright in which no mention of the use of anti-oxidants was made. However, the solutions as used, under the same conditions in all of the experiments, retained sufficient activity to cause hyperglycemia as high as 300 mgm. per cent. Since it was these values for hyperglycemia that Soskin, Priest and Schutz were comparing, and not the amounts of epinephrine or dextrose required to induce them, there seems little point to the fact that similar values for hyperglycemia might have been obtained at less cost of epinephrine, had anti-oxidants been employed.

The gradual decrease in blood flow in the experiments of Soskin, Priest and Schutz was attributable to the inherent limitations of the simple flowmeter which was used. The insertion of glass and rubber tubing into the circulatory system and the continued heparinization of the amytalized animals led to the gradual deterioration of their condition and of the circulation of blood. Although the results with both epinephrine and dextrose were subject to these same abnormal factors, it is possible that the results with each were not influenced to the same extent. It therefore seemed important to repeat these experiments, using for measurement of blood flow an adequate method, which would not interfere with the circulation. Through the coöperation of the staff of the Institute of Experimental Medicine, The Mayo Foundation, the following results were obtained, using Rein's thermostromuhr to measure the rate of blood flow. Eleven such experiments were made. The results of three of these are graphically presented as being illustrative of all.

METHODS. Dogs, from which food had been withheld for about eighteen

hours, were anesthetized with sodium amytal. A thermostromuhr unit (1) was placed as far centrally as possible on the femoral vein of one hind leg. The femoral artery of the opposite leg was ligated; through the femoral vein of this leg a long, blunt trocar needle was inserted up to the inferior vena cava, at the junction of the iliac veins, each time a sample of venous blood from the leg under observation was required. Samples of arterial blood were drawn almost simultaneously by inserting a hypodermic needle through the wall of an exposed but unobstructed carotid artery.

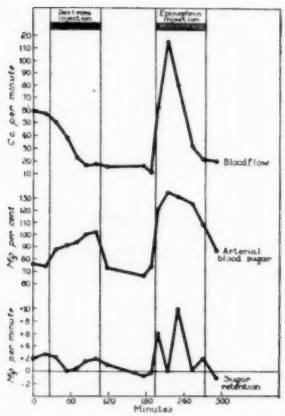


Fig. 1

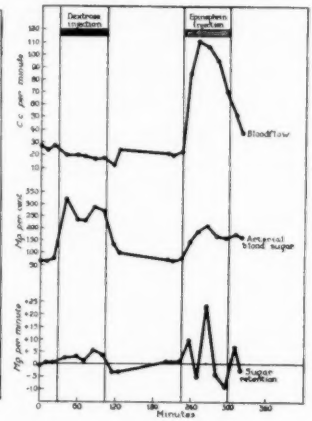


Fig. 2

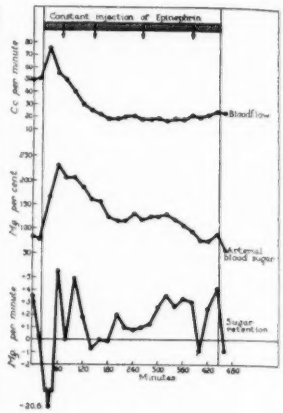


Fig. 3

Fig. 1. Comparison of the effects of the constant intravenous injection of 0.5 gram of dextrose per kilogram of body weight per hour, and of 0.12 mgm. epinephrine per kilogram of body weight per hour, respectively.

Fig. 2. Comparison of the effects of the constant intravenous injection of 1.5 gram dextrose per kilogram of body weight per hour, and of 0.12 mgm. epinephrine per kilogram of body weight per hour, respectively.

Fig. 3. Effects of a constant intravenous injection of 0.12 mgm. epinephrine per kilogram of body weight per hour, continued for seven hours. The vertical arrows in the cross-hatched bar representing the continuous injection of epinephrine, indicate the times at which the solution of epinephrine supplied to the pump was replaced by freshly made solutions.

Solutions of epinephrine and of dextrose, as indicated, were administered by constant injection with a Woodyatt pump into one of the external jugular veins. Blood sugar was determined by the method of Folin.

Results. The arteriovenous blood sugar differences varied widely throughout our experiments, depending on the glycemic level, the rate of blood flow, and other factors. As in the work of Soskin, Priest and Schutz, the passage of the blood sugar into the muscles appeared to be a reversible and cyclic phenomenon. Periods of retention of sugar by the muscles were

interspersed with periods when the blood which was leaving the muscles contained more sugar than that which was entering. Determinations of hydration of blood were not available for the present work; nor were they as important as in the work of Soskin, Priest and Schutz, in view of the more normal and rapid blood flow made possible by the use of the thermostromuhr.

When the arteriovenous blood sugar differences were correlated with the rate of blood flow through the leg and compared in terms of milligrams of sugar retained or put out per minute, not any of the eleven experiments which were made gave any evidence of decreased retention of sugar by the muscles as a result of administration of epinephrine. This was true whether the utilization of sugar under the influence of epinephrine was compared with periods of administration of dextrose, or with control periods before anything was given.

Figures 1 and 2 graphically present two experiments in which we attempted to maintain similar levels of hyperglycemia of equal duration by the constant intravenous injection of solutions of epinephrine and of dextrose respectively. In the experiment represented by figure 1 the epinephrine hyperglycemia happened to be higher than that attributable to administration of dextrose, while in that represented by figure 2 it was somewhat lower. In neither case, however, was the retention of sugar during injection of epinephrine less than that which occurred when sugar was given.

In the experiment represented by figure 3, epinephrine was constantly injected for seven hours. Our purpose was to duplicate the conditions under which, according to the indirect evidence of Colwell and Bright, "the ability to utilize glucose in any manner may be completely abolished." Our direct observations disclosed that the muscles continue to utilize sugar throughout this period. The vertical arrows in the cross-hatched bar representing the continuous injection of epinephrine indicate the times at which the solution of epinephrine supplied to the pump was replaced by freshly made solutions. It is evident that the relative smoothness of the curves of blood flow and of levels of blood sugar, in spite of the repeated change of solutions, indicates that the steady decline in both these curves is not attributable to loss in the activity of the solutions of epinephrine but to a physiologic adjustment in the animal.

SUMMARY. The use of the thermostromuhr of Rein has enabled us to obtain actual rates of blood flow, under as nearly normal conditions as possible, in the hind limbs of 11 dogs anesthetized with sodium amytal. Employing these values for blood flow to convert the arteriovenous blood sugar differences into the amounts of sugar retained by the muscles per unit of time, we have compared the utilization of sugar by the muscles during similar levels of hyperglycemia, caused by the administration of

epinephrine and of dextrose, respectively. We have obtained no evidence that epinephrine decreases the utilization of sugar by the muscles. In a similar manner we also have shown that the muscles continue to utilize sugar throughout a period of seven hours of administration of epinephrine.

The present work completely confirms the results obtained under less physiologic conditions with a simple flowmeter. The reader is referred to the paper of Soskin, Priest and Schutz for a discussion of the significance and implications of these results.

CONCLUSION

This work yielded no evidence that epinephrine decreases the utilization of sugar by the muscles.

REFERENCES

(1) Baldes, E. J., J. F. Herrick and H. E. Essex. Proc. Soc. Exper. Biol. and Med. **30:** 1109, 1933.
(2) Colwell, A. R. and E. M. Bright. This Journal **92:** 555, 1930.
(3) Cori, C. F., R. E. Fisher and G. F. Cori. This Journal **114:** 53, 1935.
(4) Folin, O. J. Biol. Chem. **82:** 83, 1929.
(5) Rein, H. Ztschr. f. Biol. **87:** 394, 1928.
(6) Soskin, S., W. S. Priest and W. J. Schutz. This Journal **108:** 107, 1934.

THE SEQUENCE OF VENTRICULAR SURFACE EXCITATION DETERMINED BY REGISTRATION OF MONOPHASIC ACTION POTENTIALS[1]

HAROLD C. WIGGERS

From the Department of Physiology, Western Reserve University, Medical School, Cleveland, Ohio

Received for publication September 23, 1936

There are three prevalent conceptions concerning the spatial and temporal aspects of ventricular excitation: 1, that the spread of excitation over the His-Tawara system and myocardial fibers occurs so rapidly that all surface points are excited essentially at the same moment (Clement, 1; Erfmann, 2); 2, that impulses are conducted over the His-Tawara system at a rapid rate to the internal surfaces of the ventricles, after which they are markedly retarded in their transit through ordinary heart muscle to the exterior, with the result that the external surfaces are excited at significantly different times (Lewis and Rothschild, 3), and 3, that the orderliness of spread is definitely related to the anatomical arrangement of muscle bundles rather than determined by eccentric pathways (Robb and Robb, 4). These widely different views are largely based upon determinations of relative times of ventricular surface negativity by various methods and consequently it ultimately becomes a question as to the method which is most reliable.

Erfmann, under the guidance of Garten, explored the ventricular surfaces of mammalian hearts by employing Clement's differential electrode, which theoretically records rather limited potential differences developed between two adjacent points. The curves obtained with such electrodes, however, coupled as they were to a string galvanometer, were exceedingly small, thus making any determination of the absolute moment of rise rather difficult.

In 1915, Lewis and Rothschild (3) introduced the *unipolar method* for exploring ventricular surfaces, the "distal" electrode being placed upon the chest, a method adopted also by C. J. Wiggers in later years. The curves obtained by using this method display a wide variety of

[1] This paper comprises part of the material contained in a dissertation submitted by the writer in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the Graduate School of Western Reserve University, June 1936. A preliminary report was presented before the Physiological Society in March, 1936, at Washington, D. C.

forms when the "exploring" electrode is shifted to different ventricular surface points. Such variations themselves suggest that the curve contours have no particular significance and certainly do not connote the changing electrical potentials occurring beneath the exploring electrode. That these obvious changes in form are partially determined by the distal and supposedly indifferent electrode is illustrated by the expedient suggested by Lewis of shifting this latter electrode whenever the curves are not of the form desired. Nevertheless it seemed probable for a while that the arrival of an excitation wave beneath the exploring electrode must cause so predominant a change in negativity that it ought to be easily distinguished in records. Lewis and Rothschild recognized the possibility that more distant changes in potential may easily be registered at the same time, but, they believed it possible to recognize such so-called *extrinsic effects* clearly. An examination of many records obtained in this and other laboratories makes it questionable, however, whether this can always be accomplished with the necessary degree of certainty. As a matter of fact, a suspicion exists that extrinsic variations may sometimes affect even the moment of the main rise and may thus obscure the actual onset of potential variation beneath the exploring electrode. Far too often the rise exhibits an angulation, in which case demarcation of the onset of negativity may well become a matter of opinion. In short, the experience gained while registering many action potentials from the mammalian cardiac surface by a variety of methods during the past three years, makes it permissible to state with a considerable degree of certainty that *no abrupt change in curves derived from the cardiac surface can be accepted as indicating the arrival of the excitation wave beneath the exploring electrode, unless the form of the entire curve does not change significantly when this electrode is moved from place to place.* Anyone who has attempted the registration of such curves with the unipolar method of Lewis and Rothschild can attest to the hopelessness of such attainment. Consequently, the sequence of ventricular surface excitation ought to be checked by other methods.

Of such other methods available, the employment of monophasic curves from a constantly localized region of injury and various uninjured spots on the ventricular surface seemed to offer some hope of success. But the registration of monophasic curves from the mammalian ventricle that are even reasonably satisfactory is a comparatively recent achievement (Schütz, 5; Jochim, Katz and Mayne, 6; and Wiggers and Wiggers, 7). The chief obstacle in the pathway to success was the production of a sufficiently localized injury which permitted neither repolarization nor pick-up from surrounding active tissue. Two very significant technical advances were made in recent years, first by Schütz, who sucked a portion of the epicardial surface into a glass tube and ligatured the nodule, thus

produced, and later by Jochim, Katz and Mayne, who created a reversible inactive-active area by applying manual pressure to one of the electrodes. In using the Schütz method for producing an injury, and a silver chloride electrode held in place by slight suction as the exploring lead, it was found by Wiggers and Wiggers (7) that the curves obtained were definitely influenced by variations of potential about the injured nodule, which in effect became merely an extension of the external lead. When this is the case, monophasic curves from the cardiac surface obviously can not be employed to study the incidence of negativity over different regions of the epicardial surface. Subsequent studies (H. C. Wiggers, 8) however offered evidence that by modifying the technique of leading, pure monophasic curves can be recorded, the upstroke of which does signal the precise moment of excitation beneath the exploring wick lead.

This communication therefore deals, first with a description of the method of registration and with the characteristics by which such pure monophasic curves can be recognized; secondly, with an analysis of errors introduced by artefacts; thirdly, with experimental evidence for the belief that the beginning of the upstroke of pure curves denotes the exact moment of arrival of the excitatory process at the exploring electrode; and finally with a presentation of results substantiating the conception that the entire ventricular surface is excited almost simultaneously as originally found by Erfmann.

Method. Essentially by trial and error, it was found that a suction electrode can be applied so that it combines the essential faculties of producing a discretely localized region of injury and of leading from it as well. It consists of a glass T-tube, tapered at one end to a 1–2 mm. bore, to the side arm of which strong suction force can be applied. The aperture of the upright extension is fitted with a rubber stopper penetrated by a non-polarizable Ag-$AgCl_2$ rod (prepared after Langelaan, 9). The tube is filled with 0.9 per cent sodium chloride solution and when suitably adjusted to the selected epicardial spot, strong suction force is applied. Precautions must be taken to prevent any loss of saline from the tube as well as to avoid the introduction of air bubbles between the tissue and the silver rod. As a result of the suction, a minute, elevated bit of tissue is drawn a short distance into the tube, the nodule being devitalized by the interference with its blood supply and by the physical stress. The color-shifts from pink → red → brown → black bear evidence of actual injury. These minute petechiae were found not to repolarize within thirty minutes after their release from the suction force. Extremely localized injury can thus be produced at almost a moment's notice.

Comparison with other methods of producing injury leads to the conclusion that for exploring punctate negativity of the ventricular surfaces, it is superior to the nodule of Schütz which on account of its size is

apt to lead potential changes from its margins and also to the manual pressure method of Katz and associates which seems to depend on pressure block rather than injury. I have found considerable difficulty in maintaining just the right degree of manual pressure for a sufficient length of time to record good monophasic curves from successive portions of the ventricular surface.

The *exploring electrode* employed for normal leads from uninjured loci is merely a glass tube the tapered end of which is threaded with cotton wick previously soaked in 0.9 per cent sodium chloride solution. Externally the wick is severed leaving a projection of approximately 2 mm. which is carefully manipulated by the fingers until flexible and cord-like. When applied with a modicum of care to a spot on the epicardial surface, it follows the movements of the ventricles exceedingly well, being of sufficient mass to retain its original setting for a long time. This electrode is also filled with normal saline solution and fitted with a prepared $Ag\text{-}AgCl_2$ rod. A similar electrode with a curved tip is used for exploring the ventricular surfaces.

The suction and exploring electrodes are both connected through a three-stage thermionic vacuum tube amplifier to a General Electric oscillograph as previously described (7). Simultaneously, and on the same film, a standard reference lead II electrocardiogram is recorded by means of a large Hindle string galvanometer. The camera paper moved at a speed of from 100 to 150 mm. per second.

Dogs averaging 15 kilos were anesthetized with morphine and sodium amytal[2] and artificial respiration was instituted, after which the sternum of each was removed to effect a convenient heart exposure.

Discussion of pure curves. Since only curves which are absolutely pure can be relied upon in determining precise excitation-times of ventricular surfaces, it is important to establish standards for their selection. These criteria are that absolutely straight lines occur between the monophasic variations, that the drop does not fall below the base line, and that no minor oscillations either precede (so-called extrinsic effects) or are superimposed upon the ascending limb or summit. Only such curves were utilized; all others obtained were discarded. Specimen records are shown in figures 2, 3 and 4. They show no pickup from extraneous sources; they display in large amplitude a clear abrupt rise of the upstroke at A and are devoid of superimposed oscillations. With these exceptions they tally with those previously described in association with C. J. Wiggers (7). There is no evidence of precedence of a positive potential. The summit is not a level plateau but a record of increasing and decreasing negativity. At the point *F* an abrupt decline of potential occurs, denoting according

[2] Thanks are due to Eli Lilly and Company, Indianapolis for a supply of sodium amytal for anesthesia purposes.

to our interpretation the precise moment at which electrical forces between the electrodes begin to return to their original potentials. The demarcation point *F* is of as great importance as the moment of rise at *A*. The *A-F* interval affords significant information as regards the changes in duration of electrical systole under variable experimental conditions. The *R-A* interval, i.e., the period between the rise of *R* of a standard lead *E.C.G.* and that of the monophasic curve enables one to determine whether experimental procedures alter this relationship.

Monophasic curves to be of value in the re-investigation of ventricular surface excitation must of necessity possess these fundamental characteristics. The most essential requirements are uniformity of contour and sharpness of onset and offset.

Sources of error in impure curves. In a previous communication with C. J. Wiggers (7) it was recorded that monophasic curves from an injured basal portion of the right ventricle and a viable point on the left showed a reduction of the period of sustained negativity (A-F interval) but no alteration in the relation of the rise, *A*, to the inception of *R* of a standard electrocardiogram when the left ventricular area was rendered ischemic by coronary occlusion. Hence the conclusion that the initial onset of such monophasic curves was due to pickup from injured areas. This conclusion was quite correct for the curves recorded but at the time we failed to recognize that these curves obtained by the method then used were impure and as briefly reported elsewhere (8) that this might modify the beginning of the main rise. The way in which such modification occurs is best shown schematically as in figure 1. *AFG* is the true curve which begins to ascend in definite relation to *QRS* as indicated by the vertical line. If preceding the rise at *A*, a potential variation is picked up from the injured region, it may give rise to positive or negative variation *X* or *X'* before the rise begins. Such effects have all the earmarks of the so-called extrinsic waves of Lewis. If time is not adequate for complete return of the curve to the base line—which is but rarely the case—the footpoint of the main rise is either retarded, *X*, or advanced, *X'*. If the pickup is of greater magnitude, the curve rises abruptly to a greater height and then begins to fall as at *Y* but when the sudden and greater change of potential is picked up by the exploring electrode, the fall—indicated by a dotted line—is prevented with the result that the curve is notched and then rises rapidly to a summit. Depending upon time relationships, this summation of two interfering electrical potentials may produce anywhere on the upstroke merely a halt, an indication of an interruption, or a large sharp oscillation. In records on slowly moving paper, minor effects may be easily overlooked. It is obvious that such impurities become serious in that the rise of the curve no longer gives a true indication of the moment of negativity represented by *A*. If on the contrary potential pickup from

the injured area occurs after the main rise has begun, the footpoint remains correct but a similar notching of the ascending limb or creation of a peak results as illustrated at *Z* in figure 1. Since there is no certain way of distinguishing between consequential and inconsequential artefacts in curve analysis, the only way of being certain that the proper point of rise is being used is to avoid altogether curves that contain them. It may be added that without doubt extraneous potentials can also be led from the exploring electrode by use of certain procedures and that these further complicate analysis; but I feel certain that the impurities in recorded monophasic curves for which investigators generally have felt inclined to apologize chiefly are due to pickup from use of an improperly injured area.

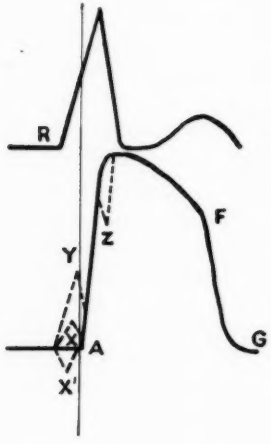


Fig. 1. Diagram indicating the ways in which potentials affecting a lead from an injured area can modify and distort the pure curve and alter the apparent footpoint. Discussion in text.

Validation of pure curves as indicators of negativity beneath an exploring electrode. In a series of 10 dogs a suction injury electrode was remotely placed on the right ventricle near the conus and an exploring electrode on the anterior left ventricular surface within a region certainly rendered ischemic by clamping a coronary vessel. Typical results are shown in figure 2. As in experiments previously reported (7), the almost immediate effect consists in a reduction of the A-F interval indicated by a reduced period of excitation, but contrary to previous results the R-A interval soon begins to lengthen, indicating that excitation is delayed. This begins to show in the second record taken 2 minutes after coronary occlusion but is more obvious in the succeeding record taken 4 minutes after occlusion. Recovery occurs after blood flow has been restored.

In five of these animals the position of electrodes was reversed, the exploring electrode being placed basally on the right ventricle and the suction electrode injured a small spot on the left ventricle to be rendered ischemic. Typical results are shown in figure 3. During a 6 minute period of ischemia neither the A-F interval nor the R-A interval shows any significant changes demonstrating that alterations of potential around the injured point have no effect on the curves.

Ventricular surface excitation times. Having established that the onset of pure monophasic curves indicates the arrival of excitation at the point contacted by the exploring electrode, all epicardial regions, both anterior

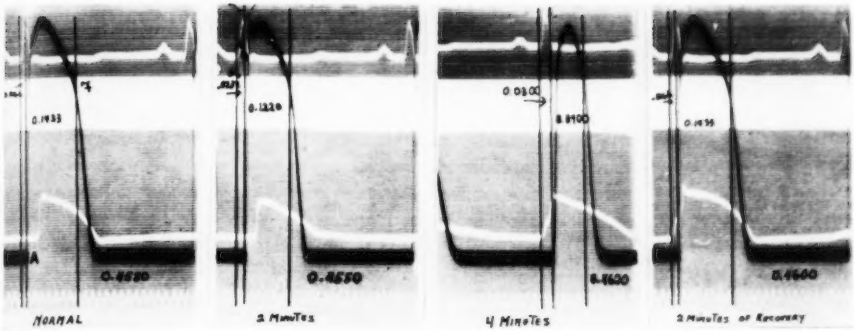


Fig. 2. Four segments of records showing above E.C.G. lead II and large monophasic curve in black. Disregard lower monophasic curve in white except to show falsification of footpoints by accidental pickup. Time in 0.02 second. Effects on A-F interval and R-A interval of coronary ligation and subsequent recovery with active exploring electrode on left ventricular area. Time intervals inscribed on records.

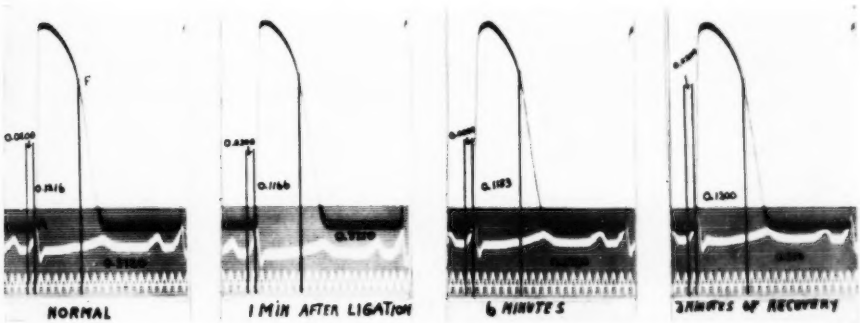


Fig. 3. Four segments of records similar to those of figure 2 taken with injury lead on left ventricle. Note absence of significant changes in comparison to figure 2.

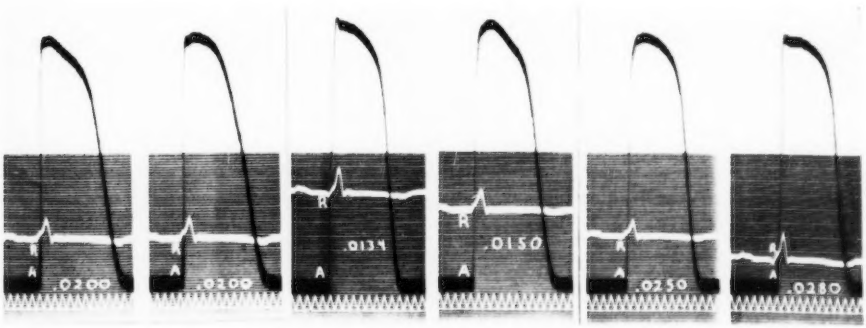


Fig. 4. Four segments of records from various portions of the ventricle showing extreme variations of the rise of the monophasic curves to R_2 of a standard E.C.G. Figures on records denote R-A intervals with parallax controlled.

and posterior, were systematically explored for the purpose of determining the relation of their negativity to the rise of R_2 of a standard electrocardiogram. The injury electrode was kept at a constant location during any series of tests but was shifted during different sets of tests on the same heart.

Figure 4 shows a series of typical curves, the first two being taken with exploring electrodes on the uninjured right ventricular surface, the third and fourth from central regions overlying the septum and the last two from the left ventricle. Curve analysis consisted in determining first the R-R intervals to assure constancy of the cardiac cycle. Then the R-A intervals were measured. Data derived from 10 such experiments were

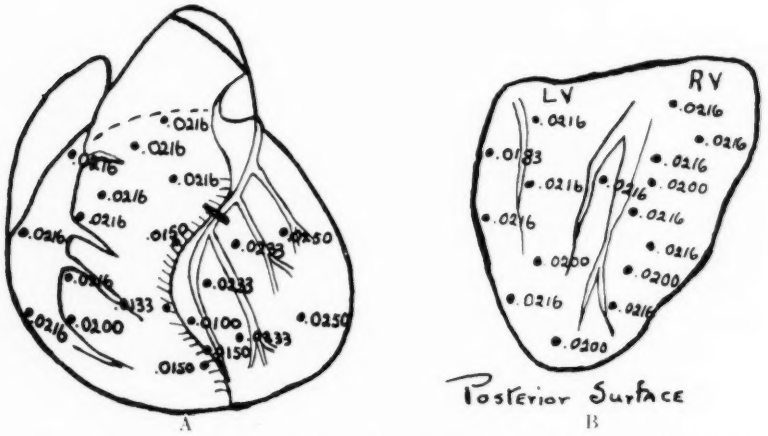


Fig. 5. Diagrams of the anterior, A, and posterior, B, surfaces of the heart showing variations in R-A intervals at different points as determined from records similar to those of figure 4.

plotted on charts of the anterior and posterior surfaces of the ventricles. Early attempts at more refined localization by photography proved superfluous owing to the small differences found. Figure 5 is an example of such plots. Each figure represents a careful determination of the R-A interval for a corresponding dot. Further to conserve space, the maximal differences in excitation times found in different regions are given in table 1. The curves, plots and tabular data presented lead to the following deductions:

1. Excepting only those figures for spots overlying the interventricular septum, the maximum time differences did not exceed 5 sigma in 84 per cent of the cases, and such discrepancies are indeed negligible as they lie within the limits of possible error in measurement. The greatest devia-

tion above this allowance in the remaining 16 per cent is one of merely 3 sigma. This is insignificant when compared to the difference of hundredths of a second reported by Lewis and Rothschild. Simultaneity of right and left ventricular excitation is the outstanding feature thereby supporting the early analysis of Erfmann and Clement.

2. A small but definite interval separates the moment of excitation of right and left ventricular surfaces, the former preceding the latter by fully 5 sigma in most cases, after the possible error of measurement has been taken into consideration. It is recalled that Lewis and Rothschild found a similar delay in the excitation of the left ventricle but it amounted to some hundredths of a second.

3. Confirming the results of Lewis and Rothschild, these time-difference measurements show that the epicardial surface immediately above the

TABLE 1

Showing maximum differences in R-A intervals in seconds

NUMBER OF EXPERIMENT	POSTERIOR SURFACE		ANTERIOR SURFACE	
	Right ventricle	Left ventricle	Right ventricle	Left ventricle
1			0.0043	0.0088
2			0.0034	0.0017
3			0.0025	0.0051
4			0.0033	0.0036
5	0.0034	0.0017	0.0053	0.0033
6	0.0015	0.0017	0.0066	0.0016
7	0.0033	0.0033	0.0034	0.0017
8	0.0050	0.0050	0.0050	0.0034
9	0.0016	0.0033	0.0016	0.0017
10	0.0016	0.0016	0.0050	0.0016

interventricular septum receives its excitation definitely ahead of the remaining right and left ventricular musculature. The discrepancy is always at least 0.01 second and frequently more nearly 0.017 second. The interval is large when contrasted to the small time differences observed between other cardiac surface points.

4. The simultaneity of excitation in all posterior spots may be pointed out, the differences between the right and left ventricles being much less marked than on the anterior surface.

Discussion. The time differences in ventricular surface negativity found by Lewis and Rothschild (3) seemed to agree with what was then known regarding the distribution of the Purkinje tissue although complete agreement as to rate of conduction in this tissue was never reached in the relatively few studies made of the question. More recently Abramson and his associates (10, 11) have presented anatomical evidence that the

Purkinje tissue is not linked to the spongy subendocardial portion in the interior of the ventricles but ramifies throughout the myocardium to within a few millimeters of the external surface. It could be inferred from these investigations that the spread of excitation through the wall of the ventricles occurs with essentially the same velocity as over the rest of the conducting system. The results now reported offer direct confirmatory evidence for such a view. Only by some such mechanism of spread at a relatively great velocity can the almost simultaneous excitation of the ventricular surface be explained. A retarded rate of spread through the ventricular wall could not be harmonized with the data.

The fact that the ventricular surfaces overlying and continuous with the septum are negative definitely earlier indicates that this large muscular mass is excited somewhat in advance and probably through passage of impulses over the A-V bundle-branches. Since no spatial sequence of surface excitation is even remotely suggested in any of the plotted charts, it seems probable that the impulses are distributed by continuations of bundle-branches to the exterior of the ventricles and improbable as believed by Robb and Robb (4) that muscle bundles constitute a supplemental route of excitation under normal conditions. Many of the contact points included in my studies were purposely placed in the general direction of muscle bundles but absolutely no sequence of excitation along points so located could be discovered. If such excitation occurs, the waves must spread at a far greater velocity than the experiments of Robb and Robb indicate.

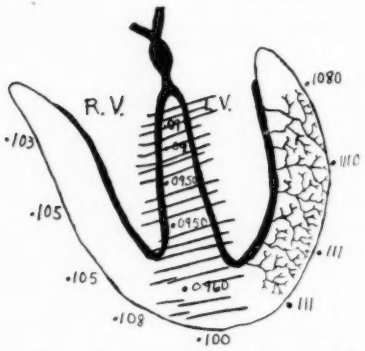


Fig. 6. Diagram illustrating coördination of surface excitation points to architecture of bundle-branches, septum and external walls. Conduction values recalculated from probable time of start from S-A node, i.e., representing P_2-A intervals.

As far as the results of surface activation are concerned there appears to be no need for revising our fundamental concept as to distribution of impulses over the His-Tawara system, first to the septum and then to the outer walls of the right and left ventricles. Our ideas regarding the velocity of eccentric spread through the ventricular walls must on the other hand be revised. The probable time differences calculated on the basis of their start from the S. A. node as illustrated in figure 6 indicate that the rate of spread both through the bundle-branches and through the walls occurs at a rather rapid rate.

SUMMARY AND CONCLUSIONS

Current conceptions regarding the spread and order of ventricular excitation seemed to merit re-investigation because the potential variations led from ventricular surfaces are apparently not as accurate as was formerly believed.

Monophasic action potentials were registered by a new technique in which a minute discrete point of injury was produced by the non-polarizable suction electrode and a lead was made from uninjured spots by a non-polarizable wick electrode. The procedure insured against pickup of potentials from the injured region which when present interfere and summate with the true monophasic curve and in so doing are very apt to render the footpoints inaccurate.

The technique of registration is given, differentiation of pure from impure curves is discussed, and the ways in which errors are introduced by such artefacts are analyzed. Experimental support is presented for the interpretation that pure monophasic curves are not affected by potentials at or near the injured point, in this method of recording. After production of left ventricular ischemia by ligation of a coronary branch, the duration of the monophasic potential is decreased and the moment of rise retarded when the exploring electrode is located in this region, whereas no alterations of the curves take place when the lead from a damaged point is taken from this area.

Pure monophasic curves were recorded successively from a large number of points on the anterior and posterior surfaces of the ventricles. The moment of rise with reference to R_2 of a standard unchanging electrocardiogram indicates the relative moments of negativity at such different spots. Curves, diagrams and a table are presented which show that the anterior and posterior ventricular surfaces are everywhere excited within 5 to 8 sigma except in the central anterior region overlying the septum which is usually excited 10 to 17 sigma earlier. No evidence of any sequence of even a small order could be discovered in points in line with muscle bundles.

The conclusion is reached that normal excitation of the ventricles occurs by transmission of impulses over the A-V bundle and its branches, first to the septum and contiguous external muscle, then to the remainder of the external ventricular wall and through them without regard to muscle bundles. Transmission through the external walls occurs at the same rapid rate at which it travels over the bundle-branches; it is not retarded as currently believed. In consequence all parts of the ventricular surfaces, excepting the central part overlying the septum are excited with a maximum time interval of about 8 sigma. Presumably such conduction occurs through recently described Purkinje tissue present throughout the ventricular walls.

I am indebted to my father, Dr. Carl J. Wiggers, for his encouragement and guidance in the prosecution of this investigation.

REFERENCES

(1) CLEMENT, E. Ztschr. f. Biol. **58:** 110, 1912.
(2) ERFMANN, W. Ztschr. f. Biol. **61:** 155, 1913.
(3) LEWIS, T. AND M. A. ROTHSCHILD. Phil. Trans. Roy. Soc. London, Part II, The ventricles, Series B **206:** 181, 1915.
(4) ROBB, J. S. AND R. C. ROBB. This Journal **115:** 43, 1936.
(5) SCHÜTZ, E. Ztschr. f. Biol. **92:** 441, 1932.
(6) JOCHIM, K., L. N. KATZ AND W. MAYNE. This Journal **111:** 177, 1935.
(7) WIGGERS, H. C. AND C. J. WIGGERS. This Journal **113:** 683, 1935.
(8) WIGGERS, H. C. Proc. Soc. Exper. Biol. and Med. **34:** 337, 1936; This Journal **116:** 162, 1936.
(9) LANGELAAN, J. W. Arch. neerl. de physiol. **14:** 592, 1929.
(10) CARDWELL, J. C. AND D. I. ABRAMSON. Am. J. Anat. **49:** 167, 1931.
(11) ABRAMSON, D. I. AND S. MARGOLIN. J. Anat. **70:** 200, 1936.
(12) ROBB, J. S. This Journal **116:** 129, 1936.

EXPERIMENTAL STUDIES ON VAGAL CONTROL OF THE FUNCTIONS OF THE LIVER

CHARLES D. SNYDER

From the Department of Physiology, The Johns Hopkins University School of Medicine

Received for publication September 30, 1936

These experiments were undertaken with the purpose of applying to the liver the newer methods of detecting autonomic nervous control.

Two species of turtle *Malaclemmys centrata* (the diamond-back terrapin) and *Chelyndra serpentina* (the common snapping turtle) were used in the experiments. After the brain and upper cord had been destroyed by pithing, the liver was prepared for perfusion through the portal vein in the usual way (see Martin and Snyder, 1922). Marriotte bottles of half liter capacities were mounted upon a platform whose vertical height above the animal can be adjusted easily with a wormscrew. The outlets from the bottles were connected by tubing to a common path and the outflow of each controlled with stopcocks. The bottles were filled with Ringer's solution buffered with $NaHCO_3$ and saturated with a 2.5 per cent CO_2 + 97.5 per cent O_2 gas mixture. A flexible gas-bag filled with the same gas-mixture was suspended above and connected to the inlet tubes of the bottles, thus supplying them with gas at atmospheric pressure as the fluid was used up. By this method the adjusted $[HCO_3^-]$ of the solutions is kept constant throughout an experiment.

Preliminary experiments with eserine revealed *a*, that, at least for fasting turtles, a concentration of eserine sulphate (Merck) in Ringer of $1:10^6$ is sufficient to "sensitize" the heart to vagal stimulation, or to a choline drug, without itself exhibiting a vagal-like action.

1. *On the action of acetyl β-methyl choline*[1] *in Ringer of low and high pH.* An animal was prepared for the purpose of testing the effect of the chloride of this parasympathomimetic drug on the minute-volume flow through the liver under constant conditions. The reservoirs having been filled with eserinized Ringer, two of these were set at pH of 7.0 and two at pH 7.5. To one of each of these groups acetyl β-methyl choline (Merck, hereafter referred to as ABMC) was added in a concentration of $1:10^5$. In the experiments the inflow tube was bifurcated so that the perfusing fluid could be sent separately through either, or both, liver and heart, inflow cannulae

[1] For reference to the earlier literature on the pharmacology of this form of choline and in conjunction with physostigmine see Hunt and Renshaw (1934); for a review of more recent literature see Alles (1934).

being inserted into the portal vein of the one and into the ascending vena cava of the other. The outflow was also arranged to pass either from the right ascending vena cava or from the systemic aorta. Finally this latter procedure was modified by putting the venous cannula directly into the hepatic vein and tying off its junction with the sinus venosus. In this latter case the rate of outflow from the liver was quite unaffected by the changing caliber of the heart chambers. The main value of this arrangement for perfusion of the heart directly, as well as the liver, is that the heart can be used at any point during the experiment to test the efficacy of any one of the perfusing solutions.

Records of the heart action by myograph were taken together with the outflow from the liver (by the drop method) on a slowly revolving drum. After conditions had become constant perfusion was allowed to proceed

TABLE 1

The effect of pH on the influence of ABMC on rate of flow through the liver

Expressed as drops per minute

INFLOW PRESSURE	RINGER + ESERINE, pH = 7.0			RINGER + ESERINE, pH = 7.5		
	Without ABMC	With ABMC	Per cent change	Without ABMC	With ABMC	Per cent change
mm.						
63	10.3	11.7	+13.5	5.2	2.3	−55.8
	7.0	8.3	+19.6			
90	14.6	15.2	+4.1	11.1	5.3	−52.2
	10.5	13.4	+28.6		6.5	
				6.2		+4.8
	10.4	13.7	+31.7	6.9	4.5	−34.8
				6.3		−28.5
	10.3					
Total mean per cent change.....			+19.5			−33.3

three minutes exactly before recording. It was found that at least six minutes were required to remove from the heart the effects of a previous perfusion of minimal effective dose of ABMC, and that no reliable measure of rate of outflow from liver can be obtained if the heart chambers intervene between hepatic vein and the outflow cannula, at least for short periods of observation. This obviously is due to the quite different effects of low and high pH on the caliber of the heart chambers (Andrus, 1919; Snyder and Andrus, 1919). The dilating effect of the choline drug on these chambers will delay the apparent outflow from the liver, due to the increased volume of fluid required for filling them. Only those records therefore in which outflow was measured directly from the hepatic vein are here considered.

As a type of these latter experiments the results of the one of March 16, 1936, are presented in table 1. The counts here recorded are for the last minute of each record, the records having continued for three minutes. The change in minute-volume output observed finally is converted into percentage with a plus sign indicating an increase; a minus sign, a decrease in volume output. The order of the readings in the table follows the order of solution change and hence that of the observations during the experiments. It will be noted that the presence of ABMC in high pH as a rule decreases; in the low pH, increases the rate of outflow from the liver, the mean change in the former case being −19.5, in the latter +33.2 per cent.

Upon comparing the action of this parasympathomimetic with that of a sympathomimetic drug on outflow from the liver of the turtle (Snyder and Martin, 1922) one is surprised to find the effects of both to be in the same direction. In both cases minimal dosage of the drugs was used. What the explanation of this may be is far from clear and will not be attempted in this place, other than to point out that the results may obtain both under general vascular constriction and dilatation as well as under localized vascular changes among a few lobules, and again under sudden permeability changes or hydration and dehydration. Further systematic study will be necessary to decide which, if any, or what combination of these processes are involved. Resort to plethysmograph is indicated, although at present this instrument seems to be inapplicable to the liver of the turtle with any degree of satisfaction

2. *Is a vagus-stuff liberated in the liver upon stimulation of the vagal nerves?* Experiments have been published by various authors which tend to show that vagal stimulation affects the carbohydrate metabolism of the liver. Snyder, Wells and Culley (1923) reported observations which indicated that vagal stimulation reduced the outflow from the terrapin liver and also the sugar output. Also upon adding pilocarpin to the perfusing solution similar results were obtained which were abolished with atropin. But thus far none of these data is highly convincing.

In the present study it was decided to allow the outflow from the liver to bathe either the heart of the same animal, whose vagal cardiac rami had been previously cut (this heart I call an *ipsicardiac test-preparation*), or, by cross circulation, the heart of another animal, which I propose to call an *aliocardiac test-preparation.*

A. *The ipsicardiac test-preparation.* In the case of the ipsicardiac test-preparation the heart is left *in situ* but isolated from its own circulation, and with its vagal rami cut. It is then perfused, as described above in section 1, with eserinized Ringer. The vagal trunks, whose branches to the liver are left intact, are prepared for tetanizing stimulation. If, upon stimulation of the vagi, vagus-stuff is liberated in the liver, then the heart

receiving the outflow from the hepatic vein ought to give evidence of its presence.

By cannulating the ascending vena cava as well as the portal vein, the perfusing eserinized fluid may be sent to either heart or liver. This was done at the beginning of an experiment, thus at once sensitizing the liver to vagal nerve stimulation, and the heart to vagal-stuff influence, and permitting the testing of the efficacy of each solution used on the heart.

The test for the complete section of all vagal branches to the heart was carried out by applying tetanizing currents to both vagi; when such stimulation of the uncut fibers had no further effect on the heart it was concluded that all the cardiac rami had been severed, whereupon one proceeded with the experiment. The tetanizing of the vagi was applied at the peripheral stumps high in the neck. The pairs of electrodes were of equal form and resistance and put in parallel in the secondary circuit of a Gaiffe inductorium. The strength of current was varied above and below that sufficient to give a sharp but not painful stimulus when the electrodes were placed on the tongue. Usually the inflow rate to the liver was kept low, 2 to 3 seconds per bubble escaping at the end of the Marriotte tube. The pH of the Ringer was set at 7.35 and otherwise made up as described in section 1. The ventricle or the auricle, or both, were connected to a muscle lever for drum recording and a combined chronograph-stimulus-signalling key, was set up to accompany the myographic tracings.

Inspection of the records shows that, after a latent period following vagal stimulation (presumably) of the liver, the heart was affected as if by a vagal-stuff that had been given off by the liver tissues to the perfusate flowing from the hepatic vein. For example, in one experiment (vi/22/26) the auricle of an ipsicardiac test-preparation, whose vagal fibers to the liver were tetanized in periods varying from 33 to 75 seconds, showed vagal-stuff effects after latent periods ranging from 3 to 50 seconds, recovery from which required periods (from the moment the stimulation began) varying from 4 to 11 minutes; the maximal negative chronotropic effects varied from 25 per cent to complete standstill of the heart.

The complete data of the experiment of vi/24/36 are given in table 2 and a reproduction of some of the drum records appears in figure 1. It will be noted in the last two lines of the table that the responses to vagal stimulation dropped out only after cutting additional vagal rami. This negative effect I believe was due, as was intended, to the cutting of the fibers supplying the liver. However, there is the possibility that the last branches of the heart only had then been severed. But against this interpretation are the facts *a*, that, at the outset when all the rami were intact, stimulation of the vagi gave prompt and complete inhibition and that after section, of what were believed to be all the cardiac rami, prolonged stimulation had no effect whatever on the heart; *b*, that the vagal-like effects, gotten (appar-

ently by the perfusates from the liver) after cutting the cardiac rami, had much longer periods of both latency and recovery than are usual as results of direct stimulation of the cardiac nerves. In addition it should be stated here that it has been found in the preliminary studies that ligation of the blood-vessels, necessary for isolated perfusion of the turtle's heart, in no way interferes with efferent vagal impulses to the heart.

The above experiments with the ipsicardiac test-preparation demon-

TABLE 2

Effect of vagal-stuff on an ipsicardiac test-preparation (behavior of right auricle to perfusate from liver)

RINGER WITH (+), OR WITHOUT (0), ESERINE	PERFUSION RATE IN DROPS PER MINUTE	TETANIZATION OF VAGI	LATENT PERIOD OF HEART RESPONSE	TIME TO RECOVER	HEART ACTION, AS RATE × STRENGTH OF BEAT		
					Before stimulus	During greatest effect	After recovery
		K.U.-minutes	*seconds*	*minutes*			
+++	20	28	6	∝	51	23	34
+++	20	30	10	∝	34	23	61
0	20	30	20	2	61	34	55
0	20	40	27	3	40	29	48
+	20	40	24	20†	49	26	49
+	20	51	28	∝	62	34	23
0	20	36	10	5	23	13	25
0	20	38	6	2	23	7	20
+	20	40	18	∝	49	15	32
0	20	40	7	8	32	6	52
0	60	40	12	6	70	18	46
0	60	50	6	5	46	4	33
0	0	50	21	8	17	1	70
0	60	50	9	10	67	15	62
+	60	50	12	2 5	65	20	48
+	60	96	51	2 5	59	29	48
+	60	50*	∝		62	60	64
+	60	100*	∝		61	64	

* In these last two cases rami below the cardiac rami had been cut before the stimulation had been applied. Tetanizing the vagal trunk has no effect whatever.

† Note that recovery is longest when eserine is present in the perfusion fluid.

strate a satisfactory method of testing vagal-stuff output of any organ of the cold blooded animal, and especially of the liver where the venous outflow empties directly into the sinus venosus.

3. *The aliocardiac test-preparation.* This is a modification of the well known methods originally employed by Loewi with frogs' hearts and by Hamburger with turtle hearts. The results of this series of experiments are illustrated by a drum record reproduced as figure 2 and by the data assembled and analyzed in table 3.

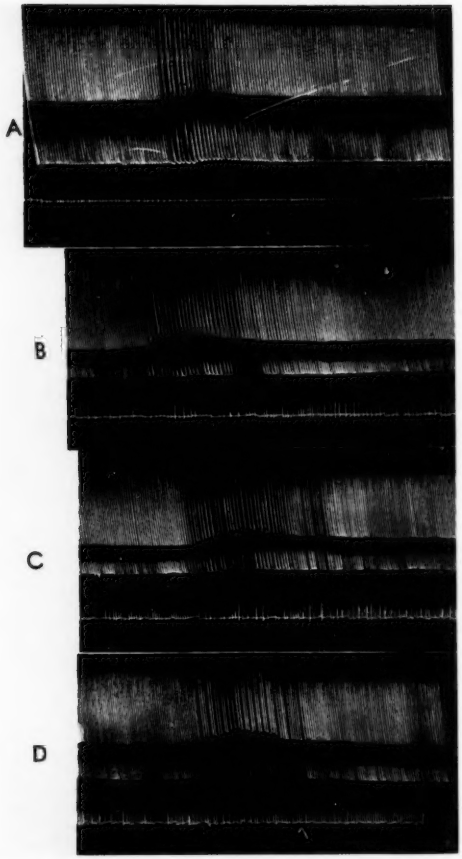


Fig. 1. Records of an *ipsicardiac test-preparation*, showing the results of four different periods of tetanizing the (liver) vagal nerves. The upper tracing is of the right auricle, the one next below is of the ventricle. The next below is of a drop-recorder registering the outflow (from the liver) as delivered by the heart through its med. aortic arch, the liver and heart being perfused in tandem at a steady head of pressure. The lowest tracing marks the time in 6-seconds intervals and also the moment of onset, and cessation of the tetanizing current.

A. Ringer without eserine. The induction coil is set at 40 Kronecker Units (K. U.). Note the positions of writing tips relative to a vertical line at the "scratch marks," and the long latent period of response to the change in liver perfusate, presumably caused by liver vagal-stuff.

B and C. Records taken two hours later; eserine has now been added to the Ringer. The stim. strength is 50 K. U. in both. Note that the latent period of the heart's response, during stimulation, is shorter than in A.

D. The stim. str. is here set at 60 K. U., but again no eserine is in the Ringer. Note again the longer latent period.

In this latter experiment the normal blood circulation of both hearts was kept intact. The liver only of terrapin 1 was prepared for perfusion as usual. The outflow from the hepatic vein, carried by cannula and tubing (about ten inches all told in length) was allowed to bathe the heart walls and collect in the pericardial sac of terrapin 2. The rate of flow through the liver was kept at 2 to 3 seconds per bubble delivered by the Marriotte inner tube. Myograms were taken on the same drum of the ventricle of terrapin 1 whose vagi were to be tetanized, and of one auricle of terrapin 2 whose heart was expected to respond to changes in the content of the liver perfusate of the first. Below the myograms on the drum records appears the trace-line of a combined chronograph (six-seconds intervals) and tetanizing stimulus-signal key. Electrical stimulation thus is shown in the records both by the signal key and by the behavior of the ventricle

Fig. 2. Record of an *auriocardiac test-preparation*; read from left to right. The upper tracing is of the ventricle, the middle one of the left auricle of the heart receiving the outflow from the perfused liver of another terrapin. The lowest tracing marks the time in 6-seconds intervals. At *1* the tetanization of the vagi to the liver was begun and the drum stopped. After the stimulus had continued 1.7 minutes (by a stop clock) the heart began to beat visibly more slowly whereupon the drum was again set in motion, and the tetanization continued for 3.3 minutes longer when it was discontinued at *2*. The perfusion rate in the liver during this time was 18 drops per minute until at *3*, when in order to hasten the recovery of the heart it was increased to 60 drops per minute; at *4* it was set again to 18 drops per minute.

(uppermost trace-line) of terrapin 1. This latter gives an excellent picture of the effectiveness of the electrical stimulus falling on the nerves.

In table 3 the data in the first and second columns give the evidence of electric stimulus gotten from the ventricle, in terms of heart-action of terrapin 1, whose liver is being both stimulated and perfused. The third and fourth columns give the evidence gotten from the auricle of terrapin 2 as it responds to the fluid flowing out of the liver of terrapin 1. The data from four trials of electrical stimulation are assembled in which it will be noted that when the tetanization of the liver was varied from 20 to 250 K.U.-Min. (Kronecker units-minutes) the maximal vagal-like effect on the test-heart varied from 16 to 40 per cent decrease in rate-strength units of heart action. (The figures for the period before stimulation and during maximal vagal-like effect on the test-heart are taken for these calculations.

Obviously this effect must be due to some vagal-like stuff given off to the fluid flowing out of the liver produced during the electrical stimulation of its nerves and confirms the findings with the ipsicardiac test-preparation.

Experiments in which the cross circulation was made quite complete (by first isolating the liver of the one animal and the heart of the other animal

TABLE 3

REMARKS	MEAN ACTION OF VENTRICLE OF TERRAPIN 1		MEAN ACTION OF AURICLE OF TEST HEART, TERRAPIN 2	
	Beats per minute	Strength of beat	Beats per minute	Strength of beat
		mm.		mm.
1. Tetanized for 16 seconds, 75 K.U.:				
During the minute preceding stimulation	13	20	42	9.0
Second minute after stimulation began	3		39	7.5
Third minute after stimulation began	11	16	38	7.7
Fifth minute after stimulation began	10	18	39	8.3
Seventh minute after stimulation began	11	20	41	9.3
2. Tetanized for 30 seconds with 50 K.U.:				
During the minute preceding stimulation	8.2	19	33	14.5
Second minute after stimulation began	0	0	34	13.5
Third minute after stimulation began	0	0	33	13.5
Fourth minute after stimulation began	6	16	37	12.5
Fifth minute after stimulation began	5.8	15.5	34	12.0
Seventh minute after stimulation began	6.2	17.5	31	12.5
3. Tetanized for 5 minutes, 50 K.U.:				
During the minute preceding stimulation	11.5	21	20	9.5
Second minute after stimulation began	0	0	21	9.5
Third minute after stimulation began	0	0	22	9.0
12th minute after stimulation began	21	18	17	7.0
14th minute after stimulation began	11.5	19	18	7.5
25th minute after stimulation began	23.5	16	16	9.0
4. Tetanized 5 minutes, 30 K.U.:				
During minute before stimulation began	40	12	18.5	10.5
First minute after stimulation began	10	16.5	18.5	8.0
Fourth minute after stimulation began	31	16.0	16.5	8.0
20th minute after stimulation began	34	9.0	19.5	9.0

from their normal blood supplies, and then connecting by tubing the hepatic vein of the former to one of the venae cavae of the latter) gave the same sort of results as those described above; only one wonders if they were exaggerated or masked by the effects of the electrical stimulation of the

vagi (as shown in sections 1 and 2 above) on the volume-minute outflow from the liver which in turn affected the filling rate of the test-heart and therefore *per se*, the filling time of the latter. To avoid such effects a modification of the technique is required which permits a gradual raising of the inflow pressure enough to maintain a steady outflow rate from the liver. In any case it is essential to have the initial inflow pressure very low, so as not to create too great a dilution of the vagal-stuff, if any, in the hepatic outflow.

SUMMARY

By isolated perfusion of the turtle's liver through its portal vein the following facts have been revealed.

1. When eserinized Ringer's solution contains acetyl β-methyl choline $1:10^{-8}$ the minute-volume outflow from the hepatic vein depends within limits on the hydrogen-ion concentration. If the pH is a little above normal the rate of flow is decreased, if below normal (pH = 7.0) then the rate, as a rule, is increased.

2. Upon simultaneous faradization of the peripheral stumps of both vagi, high in the neck, the perfusate of an eserinized Ringer (at normal pH) flowing out of the hepatic vein has a vagal-like action upon either the heart of the same animal whose vagal rami have been previously cut; or in cross-circulation (modifying the methods of Loewi and of Hamburger) upon the heart of a second turtle. In order to have distinguishing terms referring to the two methods of using the animal's own heart as test object, I have referred to them as ipsicardiac and aliocardiac test-preparations respectively.

3. Although the vagal-like stuff appearing in the hepatic outflow, following electrical tetanization of the vagal nerves has not been identified chemically, it is concluded that the results described above have increased our evidence substantially in favor of the view that the liver is innervated functionally by fibers given off from the vagal trunks.

4. Incidentally the experiments have yielded additional useful techniques for the study of chemical transmission.

REFERENCES

ALLES, G. Physiological Reviews **14:** 276, 1934.
ANDRUS, E. C. This Journal **48:** 221, 1919.
HUNT AND RENSHAW. J. Pharmacol. and Exper. Therap. **51:** 237, 1934.
SNYDER AND ANDRUS. Ibid. **14:** 1, 1919.
SNYDER AND MARTIN. This Journal **62:** 185, 1922.
SNYDER, WELLS AND CULLEY. Ibid. **66:** 485, 1923.

THE FLOW AND PROTEIN CONTENT OF SUBCUTANEOUS LYMPH IN DOGS OF DIFFERENT AGES[1]

RUSSELL HOLMAN

From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City

Received for publication October 3, 1936

The following represents an attempt to correlate capillary permeability with age, by determining the flow and protein content of lymph obtained from cannulation of subcutaneous lymphatic trunks in the extremities of dogs (Drinker technique).

METHODS. Normal healthy mongrel dogs ranging in age from 3 months to about 15 years, and in weight from 4 to 24 kilos, and selected for grouping into "pups," "young adult dogs," and "old dogs," were used in these experiments. They were last fed an ample quantity of kennel diet about 24 hours before operation. Water was removed from the cage at 9:00 o'clock in the morning, and since all operations were performed in the afternoon, all dogs had been without water for 4 to 6 hours. From 200 to 500 cc. of water, depending upon the weight of the animal, were then administered by stomach tube. This procedure was carried out to favor a fairly uniform ample state of hydration.

The technique described by Drinker (1) was used in cannulating the lymphatic trunks. In order to avoid possible alteration of the lymph during its passages through lymph nodes and to keep conditions uniform, the operative site was always over the joint corresponding to the wrist or ankle. No differences between fore and hind feet have been encountered in these experiments. Local anesthesia (1 per cent novocain without adrenalin) was used in all experiments. In each age group, lymph was collected under the following conditions in roughly the same percentage of experiments: 1, passive motion; 2, normal walking about the laboratory, and 3, walking on a motor-driven treadmill at a constant rate of 2 to 3 miles per hour. Since no striking differences resulted from the use of these various methods, all experiments in each age group have been considered collectively. Lymph flow is recorded as that collected from a single lymphatic trunk in cc./hr. for the total duration of the experiment which varied from 45 minutes to 3 hours, the variations being about equally divided between the different age groups. Lymph protein is listed as the average of all analyses, if more than one was done.

[1] This investigation has been aided by a grant from the Josiah Macy, Jr. Foundation.

The micro-Kjeldahl method (4) was used for all protein analyses, which were run in duplicate if the quantity of lymph permitted. Usually N.P.N. was determined only on the blood plasma, collected at the end of the experiment, and the figure so obtained subtracted from the total nitrogen of the lymph. Many experiments have shown the N.P.N. of the two fluids to be practically identical. In a few experiments in which lymph flow was small and no analyses were made on the blood plasma, N.P.N. was not determined but was estimated at 30 mgm./100 cc., which is about the average of all analyses under the conditions outlined above.

TABLE 1

*The flow and protein content of lymph collected simultaneously from two lymphatics in the same leg**

DOG	AGE	WEIGHT	LEG USED	LYMPHATIC TRUNK	PERIOD A		PERIOD B		PERIOD C	
					Flow	Protein	Flow	Protein	Flow	Protein
		kgm.			*cc. per hour*	*grams per 100 cc.*	*cc. per hour*	*grams per 100 cc.*	*cc. per hour*	*grams per 100 cc.*
35-6	5½ mo.	13.9	R. H.	L	3.00	1.52	2.60	1.85	2.41	1.21
				M	2.13	1.51	1.30	1.62	1.13	1.29
	6 mo.	16.0	L. H.	L	1.40	1.02	0.40	2.04	0.50	1.53
				M	1.35	0.82	0.75	2.21	0.80	1.84
34-795	3¼ yr.	19.0	R. H.	L	0.49	1.44	0.25	1.99		
				M	0.15	1.24	0.10			
	3½ yr.	18.3	L. H.†	L	0.40	2.21	0.40	1.97	0.15	2.08
				M	0.45	2.29	0.70	2.10	0.35	2.15
35-656	±15 yr.	17.9	L. F.	L	1.10	1.06	0.90	1.27		
				M	0.60	0.96	0.45	1.65		
		17.4	R. F.	L	0.30	1.04	0.32	1.65		
				M	2.10	0.86	0.60	1.27		

Abbreviations: R. H. = Right hind leg; L. H. = Left hind leg; R. F. = Right fore leg; L. F. = Left fore leg; L = Lateral to main vein; M = Medial to main vein.

* Passive motion and massage used in all experiments.

† A lymphatic trunk in this leg was previously cannulated 6 months before this experiment. Between period A and period B several collateral channels (which become more numerous after such an operation) were ligated.

EXPERIMENTAL OBSERVATIONS. As a check on the methods, determinations were made of the flow and protein content of lymph collected simultaneously from two lymphatic trunks in the same extremity during 2 or 3 half-hour periods. The results of 6 such experiments on corresponding legs of three dogs, one in each age group, are listed in table 1. Although the protein content of the lymph obtained from the two trunks was usually of the same order of magnitude, occasional samples were encountered that varied as much as 23 per cent (dog 35-656, L. F., Per B). Variations in

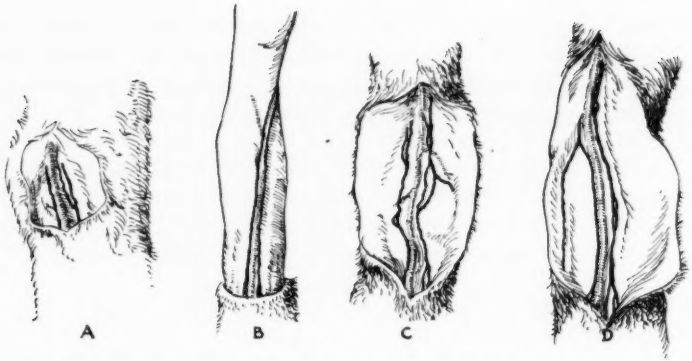


Fig. 1. A, C, and D fore legs, B hind leg. All diagrams at "wrist" or "ankle" joint.

TABLE 2

Lymph flow in dogs of different ages

AGE GROUP	NUMBER OF ANIMALS	NUMBER OF EXPERIMENTS	LYMPH FLOW			
			Mean	Extremes	Standard of deviation	Probable error of mean
			cc. per hour	*cc. per hour*	*cc. per hour*	*cc. per hour*
Pups	11	22	1.57	0.60–4.30	0.81	0.12
Adult dogs	17	34	1.34	0.20–5.77	1.00	0.12
Old dogs	10	20	1.65	0.80–3.10	0.59	0.09
All dogs	38	76	1.49	0.20–5.77	0.86	0.07

TABLE 3

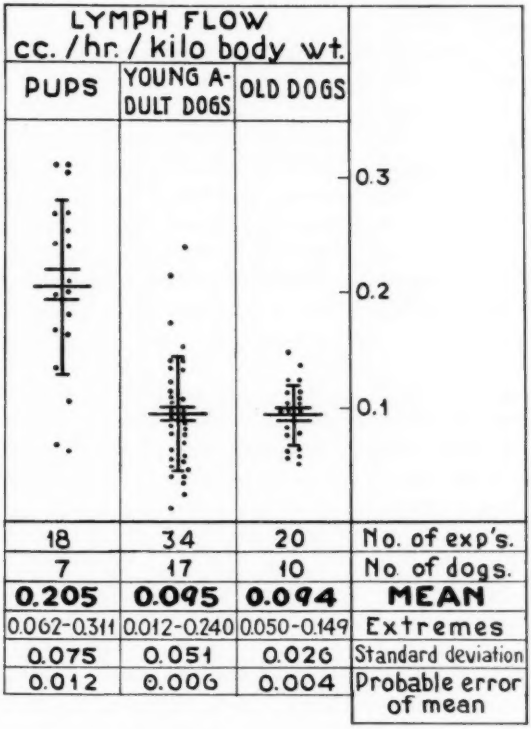
Lymph protein in dogs of different ages

AGE GROUP	NUMBER OF ANIMALS	NUMBER OF EXPERIMENTS	LYMPH PROTEIN			
			Mean	Extremes	Standard of deviation	Probable error of mean
			grams per 100 cc.	*grams per 100 cc.*	*grams per 100 cc.*	*grams per 100 cc.*
Pups	11	21	1.48	0.44–2.59	0.47	0.070
Young adult dogs	6	11	1.49	0.66–2.61	0.58	0.123
Old dogs	7	11	1.40	0.83–2.41	0.42	0.089
All dogs	24	43	1.46	0.44–2.61	0.50	0.051

lymph flow were considerably greater and in one instance amounted to 700 per cent (dog 35-656, R. F., Per A). Sacrifice experiments after injecting

subcutaneously between each of the toes a poorly diffusible dye such as Chicago Blue dissolved in dog serum showed that these variations in lymph flow could be accounted for, in part at least, by the anatomical variations in and anastamoses between the subcutaneous lymphatic trunks. Some of these anatomical variations are pictured in figure 1.

The results of these preliminary experiments clearly indicated that any answer to the question of a correlation between age and the flow and/or



18	34	20	No. of exp's.
7	17	10	No. of dogs.
0.205	**0.095**	**0.094**	**MEAN**
0.062-0.311	0.012-0.240	0.050-0.149	Extremes
0.075	0.051	0.026	Standard deviation
0.012	0.006	0.004	Probable error of mean

Fig. 2. $\frac{\text{Lymph flow}}{\text{Body weight}}$ in dogs of different ages

protein content of lymph must depend upon the statistical analysis of a significant number of observations in each age group. Such analyses for lymph flow are given in table 2 and for lymph protein in table 3. It will be seen that there are no significant differences in either table, and this despite the marked differences in body weight in the different age groups. On theoretical grounds, the larger the dog the larger the foot, and the larger the foot the greater will be the amount of lymph collected. It is well known, however, that different breeds of dogs have relatively larger feet

than others, and since all of the dogs used in these experiments were of mongrel extraction, the only way this theoretical consideration could be taken into account was to introduce a correlation factor for the entire weight of the animal. When this is done lymph flow becomes a little over two times as great in growing animals as in adult animals, but once maturity has been reached there is no significant change even in very old dogs (fig. 2).

Discussion. The suggestion of Drinker and Field (2) that some of the objective changes associated with advancing years may be due to a decrease in capillary permeability which is only partially compensated by an increase in B.P. has not been borne out by these studies. Instead, the results of these studies indicate that capillary permeability is increased during growth and development, but that after the attainment of maturity there is no significant change from the lower "maintenance" level established at that time. It is interesting in this connection that Lowrey (3) has recalculated the data of Thomas to show that the amount of water in the skin of dogs decreases from 79 per cent in the newborn to about 50 per cent in the adult. Possibly the relatively greater lymph flow observed in pups is no more than an expression of this phenomenon.

SUMMARY

In an attempt to correlate capillary permeability with age the flow and protein content of subcutaneous lymph was determined in normal dogs of different age groups: "pups," "young adult dogs," and "old dogs."

There was no significant difference in lymph protein in the different age groups.

When a correlation factor is introduced for the weight of the animals lymph flow is about twice as great in growing animals, but once maturity has been reached there is no significant change even in very old dogs.

The question as to whether this relatively greater lymph flow in pups is due to increased capillary permeability or is merely an expression of the relatively greater content of body water in growing animals is left open.

Drinker's suggestion of a decrease in capillary permeability associated with advancing years (2) has not been substantiated.

REFERENCES

(1) Drinker, C. K. and M. E. Field. Lymphatics, lymph and tissue fluid. The Williams & Wilkins Co., Baltimore, Md., 1933.

(2) Drinker, C. K. and M. E. Field. This Journal **97:** 32, 1931.

(3) Lowrey, L. G. Anat. Record **7:** 143, 1913.

(4) Peters, J. P. and D. D. Van Slyke. Quantitative clinical chemistry. Vol. II, Methods. The Williams & Wilkins Co., Baltimore, Md., 1931.

SOUNDS DUE TO MUSCULAR CONTRACTION AND THEIR IMPORTANCE IN AUSCULTATORY QUALITIES OF THE FIRST HEART SOUND[1]

RICHARD W. ECKSTEIN

From the Department of Physiology, Western Reserve University Medical School, Cleveland, O.

Received for publication October 5, 1936

It is generally accepted that sounds are created by contraction of skeletal as well as of cardiac muscle and that such a component enters into the first heart sound. Indeed some clinicians are of the opinion that, by virtue of such a component, hyperdynamic and hypodynamic actions of the ventricles can be detected by changes in the intensities and qualities of the first sound. The idea that muscular contraction causes audible vibrations has however been questioned from time to time, and most recently by W. Dock, Jr. (1).

The practical aspects of the problem are separable into several questions. Does contracting skeletal and heart muscle evoke sound vibrations under modes of contraction probable in the body? If so are such vibrations of sufficient intensity to enter into the composition of the first sound or are they obscured by other elements? Does diminished contractile power or abrogation of contraction in a section of heart, e.g., following coronary occlusion, lead to modifications in sounds?

Sounds due to skeletal muscle contraction. A first approach to the problem was made by recording sounds from the gastrocnemius muscle of a frog contracting *a*, under various degrees of isometricity up to complete abolition of shortening, and *b*, during progressive stages of fatigue. The muscles were stimulated with single break shocks and the contractions recorded with a calibrated optical torsion myograph which allowed a calculation of the amount of shortening. The myograph employed is described by Wiggers (2). The degree of shortening can easily be controlled by increasing the initial tension under which the muscle is attached.

The direct method of recording sounds was used, the apparatus consist-

[1] This paper comprises part of the material contained in a dissertation submitted by the writer in partial fulfillment of the requirements for the degree of Master of Arts to the Graduate School of Western Reserve University, June 1936. A preliminary report was presented before the Physiological Society in March 1936 at Washington, D. C.

ing of a modified Wiggers-Dean recorder (3) and a sound receiver connected by a heavy rubber tube 20 cm. long and 5 mm. internal diameter. A cross-sectional view is shown in figure 1. The modifications of the recorder were made in accordance with Frank's principles and eliminated certain disadvantages that have heretofore restricted its use. They resulted in *a*, making a more permanent membrane capable of being stretched tighter and therefore having a higher frequency; *b*, increasing the sensitivity by adopting the new principle of light projection suggested by Hamilton, Brewer and Brotman (4); *c*, increasing the decrement of its free movement by increasing its damping, and *d*, eliminating impacts more completely thus producing a straight line between the sounds.

The rubber cement membrane was made by passing the segment capsule through a thin film of rubber cement. Immediately the capsule was placed in a capsule holder and lowered into a small bottle of sulfur chloride

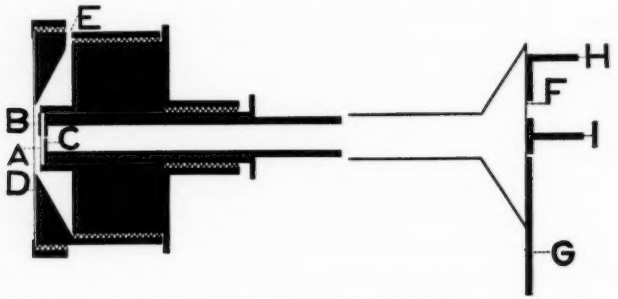


Fig. 1. Diagram of modified direct sound recorder (left, and receiver (right). *A*, vulcanized rubber cement membrane; *B*, mirror; *C*, damping plate; *D*, glass front; *E*, opening for equalizing pressures; *F*, tense rubber dam closing receiver; *G*, *H*, *I*, different types of bamboo for sound "pickup" used in different types of experiments.

and the membrane held just above the level of the fluid for a period of 5 seconds. It was then lifted out and the membrane permitted to dry for a few seconds depending upon its thickness. While the membrane was drying, the stretch was roughly determined by blowing over its surface. If it appeared too loose it could still be stretched by pulling it over the edge of the capsule with the thumb. Usually the sulfur chloride increased its tension sufficiently. Such a membrane often lasted two weeks and longer, and could be handled without the former danger of breaking.

The sensitivity was increased by projecting light beams at least two to three meters by the use of a 0.750 or 1 D. plano-convex mirror suggested by Hamilton, Brewer and Brotman (4). The mirror was flooded with a broad beam of light from a 500 W. tungsten filament lamp without lenses and the reflected light focused on the camera. With such an arrangement the side tube could generally be left completely open thus eliminating coarser

vibrations due to impacts. The periodicity of the system was reduced by use of damping plates, one behind and another in front of the membrane. One millimeter behind the membrane was placed a metal plate pierced

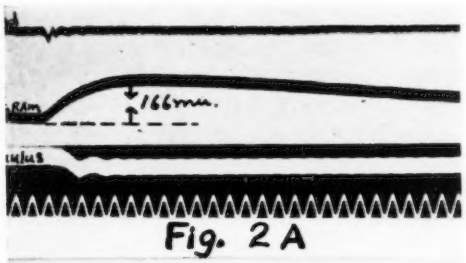


Fig. 2 A

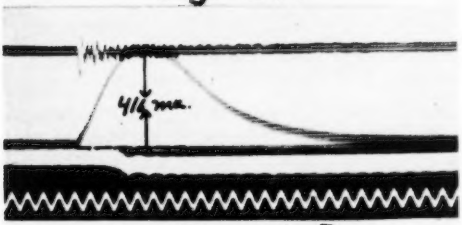


Fig. 2 B

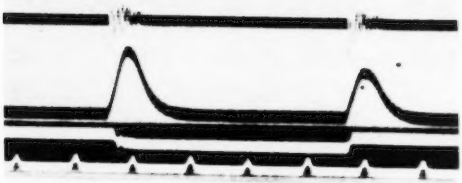


Fig. 3 A

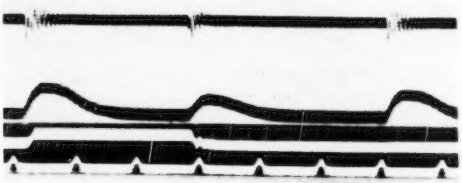


Fig. 3 B

by an opening 0.026 inch in diameter. The glass window in front of the membrane was moved back to a distance of one millimeter from the mirror thereby damping the membrane both in front and behind.

The receiver consisted of a circular capsule. It was covered by a tightly stretched rubber dam to which was securely cemented either a short bamboo lever or an L or T-shaped rod of light wood (fig. 1), each containing a small hole at the end by which it could be firmly sewed to the muscle or to the tendon near its long attachment. The frequency of the entire system ranged from 290 to 350 vibrations per second. It was still periodic but a further degree of damping was found to be unnecessary, since the frequency was high in comparison to that of the vibrations recorded.

Results. Figure 2 shows typical records in each of which the upper curve represents the vibrations and the lower curve the amount of shortening. In record A the muscle shortened 166 micra with the production of small vibrations during the first part of contraction. Record B shows shortening of 416 micra with large vibrations occurring during contraction and smaller vibrations occurring even during the first part of relaxation.

Figure 3A shows different size contractions following weak break and make shocks. The amplitude and number of vibrations of the sounds are directly related to the extent of contractions. This is further illustrated by records of figure 3B which were taken during fatigue resulting from perpetual stimulation of the same muscle. It can be seen however that the duration of the sound vibrations is also governed by the duration of contraction.

These curves serve as examples of a large number of tests which permit the conclusions 1, that sounds are only produced when a critical minimal degree of shortening occurs; 2, that the amplitude of vibrations is proportional to the degree of shortening, and 3, that the duration of the vibrations is dependent both on amplitude and duration of the contraction process.

A careful examination of the various records furthermore shows that the vibrations quite often are introduced by an oscillation at the very beginning of contraction which is usually in the same direction and similar to that seen in the first heart sound. This is followed by a series of larger vibrations progressively increasing in amplitude which end just before the peak of contraction is reached. These in turn are succeeded by a series of smaller vibrations occurring at the peak of contraction and often during the first part of relaxation. They are of irregular periods and amplitudes. It is apparent that they have their origin in the muscle and not in the system since their periods range from 8 to 12 sigma while that of the system is 3 sigma.

Such vibrations are not friction sounds arising between the muscle and the lever since the lever was stitched tightly to the muscle. Even when the lever was stitched to the immovable tendon near its bony attachment, small vibrations still occurred. In such cases there could have been no movement as was proven by completely closing the side tube and observing

no slower deflection of the light beam. This demonstrates that the vibrations are transmitted through tendonous structures. Slow pressure changes in the system played no part in the sounds recorded since the side tube was left completely open. If mechanical changes had occurred the vibrations would have been on either side of a line which moved up or down as the muscle contracted or relaxed, but the records show vibrations on either side of a straight line.

Since such vibrations have frequencies well within the range of the human ear and since they can also be heard by substituting a stethoscope for the recorder, they must be interpreted as sounds. The fact that they occur only when some shortening is permitted seems to support the idea that they have their origin in the friction between muscle fibers which certainly would be greatest when some shortening occurs.

The conclusion is reached therefore that the contraction of skeletal muscle, even when shortening is insignificant, produces sounds of frequencies corresponding with those of recorded heart sounds.

Sounds due to contracting heart muscle. Since the histological structure of cardiac muscle differs essentially from that of skeletal muscle, the question must still be raised whether demonstration of sounds from twitch contraction of skeletal muscle proves existence of sound during the contraction of heart muscle.

To study the question in mammalian hearts, it was necessary to devise means for eliminating valve closure and pressure changes in the ventricles and yet keep the muscle contracting. Several progressive experiments were therefore carried out on cats' hearts perfused with Locke's solution by the Langendorff method. The heart was removed from a cat immediately after death and the coronary vessels perfused from a cannula placed in the aorta, but outside of the aortic valves. Both sounds could easily be heard or recorded by stitching the receiving stylus to a part of the ventricle. To eliminate the effects of intraventricular pressure changes and valve closure a "reduced ventricular preparation" was made. While the heart continued to beat, the right coronary and the left circumflex branches were ligated taking care to retain the aortic valves. All the ventricular musculature was then cut away except a V-shaped strip surrounding the left ramus descendens. The weight of this strip averaged about 6 grams. After ligation of the leaking small vessels, the strip continued to beat and with the occasional help of epinephrine the beat could be made forceful.

When the receiver was sewed to such a strip of ventricular muscle devoid of valves, chordae tendineae and ventricular chambers, vibrations were still heard and recorded during contraction as illustrated in figure 4. It made no difference whether the muscle was or was not under a slight degree of tension. The side tube was always left open but at times it was necessary to add a very short length of tube to render the apparatus sensitive enough.

The vibrations show irregular frequencies and amplitudes with period ranging from 14 to 50 sigma which are far above those of the system and they therefore must originate in the cardiac muscle and not in the system.

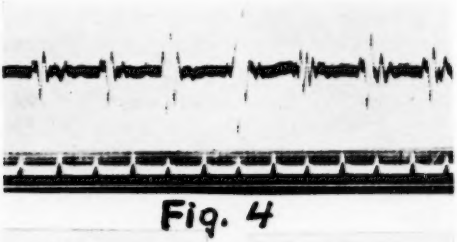


Fig. 4

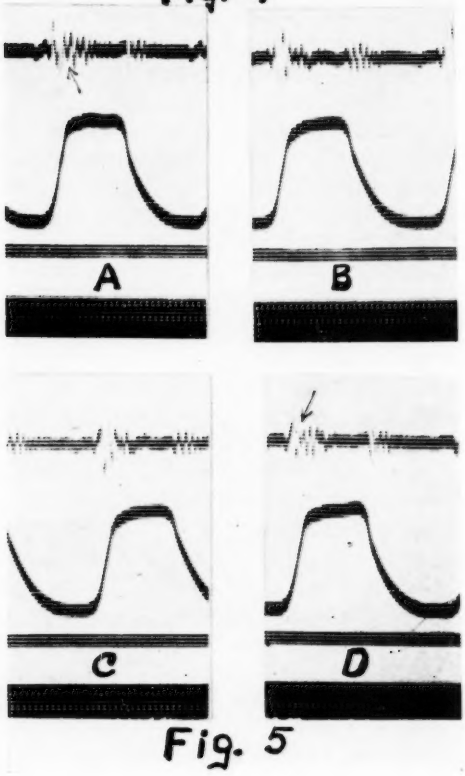


Fig. 5

The record shows fewer vibrations than are seen in a normal first heart sound. When heard with a stethoscope such vibrations were similar to those of a feeble first heart sound. Since the vibrations increased in

amplitude and number after epinephrine, it is true as in skeletal muscle that they depend upon the vigor of contraction. The conclusion then is reached that contraction of cardiac muscle in itself is capable of producing vibrations similar to those of the first heart sound.

The effect of localized ischemic abolition of contraction upon sound records from exposed hearts. While preceding experiments leave no doubt that skeletal and cardiac muscle *per se* can produce sounds which vary in intensity and duration with the vigor of contraction, the question still remains whether they participate in the irregular vibrations heard and recorded over the chest wall or directly from the heart, and whether changes in intensity of contraction significantly modify the amplitude, number, and form of the component vibrations of the first sound. Since Tennant and Wiggers (5) have shown that an ischemic area fails to contract within one minute after coronary ligation and since they have shown that this may be so nicely compensated that intraventricular pressure is not altered, it was conceived that the question might be elucidated by recording sounds directly from such an area before and after coronary ligation.

The results obtained were not as crucial as hoped for and will therefore be reported only very briefly. After exposure of the heart and attachment of the recorder lever (I, fig. 1) intraventricular pressures were recorded by use of a hypodermic needle manometer similar to that of Hamilton et al. (4) but modified in certain respects by Gregg (6). After control sound and pressure records had been taken, the ramus descendens was occluded for 4 to 6 minutes and then released. Figure 5 shows the intraventricular pressure curves and sounds at various intervals after such ligation.

Figure 5A represents a control record. After ligation, intraventricular pressures declined slightly at first but soon recovered to normal in the majority of cases. Segment B shows a record of sounds and pressure 4 minutes after coronary ligation. The amplitude of the pressure is essentially equal to that of segment A but the form of the summit is slightly different. Segment C taken 6 minutes after coronary ligation shows a pressure curve, that has a similar form but is of slightly smaller amplitude. Segment D is a control taken about 1 minute after restoration of the coronary circulation. As to changes in heart sounds, one must be less definite. Careful study of sounds by enlargement and registration on more rapid paper often but not always revealed the presence of a series of rapid vibrations barely discernible at points indicated by the arrows in segments A and D. When present they seemed to disappear when muscle ceased contracting after coronary ligation as in segments B and C. Quite as often, new groups of rapid vibrations seemed to be introduced at about the same times during the period of ischemia. We hesitate therefore to attribute any significance to them. The amplitude and duration of the vibration groups comprising the first sound show no essential difference except

during the stages when intraventricular pressure was decreased temporarily.

These results therefore offer no support for the conclusion that abolition of muscular contraction significantly affects the character of sounds even when led from muscular regions immediately involved, provided the height and form of the intraventricular pressure remains unaltered. However it should be remembered that such ischemic muscle is actually stretching 8–10 times as much as it normally shortens. It is quite conceivable that such periodic stretching may produce as many or more sound vibrations than are produced by normal contraction. Furthermore the smaller and slower vibrations arising directly in the muscle may well be obscured by the larger and more frequent vibrations arising in other structures in consequence of the sudden elevation of intraventricular pressure. In all records obtained, the intensity and duration of the first sound seems to be definitely correlated with the steepness and height of left ventricular pressure as Wiggers (7) has pointed out.

If any conclusion is permissible from an assembly of these experiments, it would be that despite the fact that contracting ventricular muscle causes sound vibrations, they probably do not constitute an essential part of the first sound heard or recorded from the chest wall or even directly from the heart. There is little ground for any belief that sudden abolition of contraction following coronary occlusion or any hypodynamic action in myocardial failure can directly modify sounds heard on auscultation sufficiently to be of diagnostic value.

These conclusions do not affect the premises of Wiggers (7), again supported in these experiments, that the vigor of muscular contraction can modify the amplitude, number, and perhaps the periods of vibrations indirectly, i.e., by causing structures including the valves, chordae tendineae, ventricular walls (contracting or not contracting) to vibrate in proportion to the suddenness and height to which intraventricular pressure increases. It is immaterial however whether this is accomplished through participation of all fibers or through deletion of some and compensatory increase in contraction of others. Consequently there seems to be little hope that auscultation or registration of heart sounds can be of service in the diagnosis of coronary infarction.

SUMMARY AND CONCLUSIONS

The question as to whether skeletal muscle of frogs and isolated portions of the cats' ventricles give rise to sounds when contracting under varying degrees of isometricity was reinvestigated by registering vibrations from such muscles. For this purpose the Wiggers-Dean capsules were so modified as to increase their sensitivity, decrease their periodicity and enhance the stability of the rubber cement film.

The results showed 1, that sound vibrations can be heard and recorded from such preparations provided that a minimal degree of shortening occurs, and 2, that the number, amplitude and duration of these vibrations depend upon the vigor of contraction and the degree of shortening permitted.

The investigations were then extended to registration of vibrations from the anterior surface of the dog's ventricles before and after ligation of the ramus descendens. This enabled a comparison of sound vibrations from a region that is first contracting and then passively stretched during systole. No significant changes could be detected as long as intraventricular pressure curves remained essentially unchanged as to gradients and magnitude.

The conclusion is drawn that vibrations produced directly by contraction are probably obscured or submerged by the more intense vibrations of other structures and that they are related most definitely to changes in intraventricular tension. Consequently there seems little probability that alterations in recorded first sounds can be of help in the diagnosis of coronary infarction.

I desire to acknowledge the advice and help of Drs. Carl J. Wiggers and Donald E. Gregg in the prosecution of this investigation.

REFERENCES

(1) Dock, Jr. Arch. Int. Med. **51**: 737, 1933.
(2) Wiggers. Physiology in health and disease. Lea & Febiger, Philadelphia, 2nd ed., p. 42, 1937.
(3) Wiggers and Dean. This Journal **42**: 476, 1917.
(4) Hamilton, Brewer and Brotman. This Journal **107**: 427, 1933.
(5) Tennant and Wiggers. This Journal **112**: 351, 1935.
(6) Gregg. This Journal **118**: 399, 1937.
(7) Wiggers: Arch. Int. Med. **24**: 471, 1919.

FRACTIONATION OF THE OUTPUT OF THE HEART AND OF THE OXYGEN CONSUMPTION OF NORMAL UNANESTHETIZED DOGS[1]

SANFORD E. LEVY AND ALFRED BLALOCK

From the Department of Surgery of Vanderbilt University, Nashville, Tennessee

Received for publication October 10, 1936

A method for the determination of the blood flow and oxygen consumption of the individual organs of the intact unanesthetized animal has not been devised. The thermo-electric stromuhr has been used with success for the determination of the flow of blood through a number of organs but this method does not include a means for the taking of samples of blood. However, a procedure for the determination of the blood flow and oxygen consumption of the kidney was devised recently by Mason, Blalock and Harrison (1936), and a similar method for the liver was described by Blalock and Mason (1936). By employing these two methods the caval drainage of the portal system and kidney can be determined. Also susceptible to measurement by an adaptation of the method is the flow of blood in the inferior vena cava below the renal veins, coming largely from the lower extremities, and the flow of blood in the superior vena cava above the right auricle, coming mainly from the upper extremities and head.

The purpose of the present experiments was to determine the flow of blood and the oxygen consumption of the kidneys, posterior extremities and pelvic organs, upper extremities and head, and the portal system, and to compare these determinations with the total cardiac output and oxygen consumption.

METHOD. Dogs were used as the experimental animals in all instances. They were trained to lie quietly on the table. No anesthetic was used except for the local injection of novocain over the external jugular vein at the site where the cannula was introduced. The animals gave no evidence of pain during the experiments and were observed to eat or play following the completion of the studies. One animal received an injection of morphine preliminary to the studies. No food was allowed for eighteen hours preceding the experiment. Heparin was used as the anti-coagulant.

The principle of the method for determining the blood flow has been described previously. In brief, it consists of inserting into the external

[1] Aided by a grant from the Division of Medical Sciences of the Rockefeller Foundation.

jugular vein a metal cannula equipped with two externally expanding balloons. One balloon is located just above the closed distal end, and the second balloon is 7.5 cm. above the first. Multiple perforations into the lumen of the cannula are present between the balloons.

The flow of blood from the posterior part of the body was determined by passing the cannula until the upper balloon was below the level of the entrances of the renal veins. The position of the renal veins had been determined during the course of a previous operation at which time several small veins in the neighborhood of the renals were ligated, as well as the spermatic or ovarian veins. The upper balloon was then inflated and the quantity of blood flowing through the cannula was determined. The kidney flow was determined after having placed the cannula so that the lower balloon was below and the upper balloon above the renal veins. Both balloons were inflated during the measurement. In a few instances in which very short dogs were used, the flow through the posterior extremities was determined by subtracting the renal flow from the combined figure for the kidneys and posterior extremities. The latter figure was obtained by determining the flow when only the upper balloon was inflated, the cannula being in the position for the kidney flow determination. In determining the flow of blood through the hepatic veins, the cannula was placed with the aid of a fluoroscope so that the upper balloon was seen to be just above the diaphragm. Both balloons were inflated. The flow of blood through the upper extremities and head was determined by withdrawing the cannula until the lower balloon was in the superior cava just above the heart. The lower balloon was then inflated and the flow determined. The level at which the outflow tube was placed during the measurement has been considered previously.

Immediately following the measurement of the flow through the various parts and before the blood was replaced, a sample of blood was withdrawn from the tube in order that the oxygen content might be determined. Arterial blood was obtained from the femoral artery by puncture. The oxygen consumption of the part was calculated by multiplying the flow of blood per minute by the arteriovenous difference in oxygen content.

The cardiac output was determined by the use of the Fick principle. Mixed venous blood was obtained in some instances by puncture with a needle and in others it was withdrawn from a cannula which was placed in the right side of the heart through the external jugular vein. A control study of the cardiac output was carried out several days before the experiment proper was performed. In six of the experiments, the output of the heart was determined immediately before and after the measurement of the flow through the various parts. The oxygen consumption of the body was measured by the use of a Benedict spirometer. It was measured continuously when the animal was breathing oxygen through the experi-

ment. Otherwise, it was measured before and after the studies on the rate of blood flow.

Results. A total of thirteen experiments were performed and the results are given in Table 1. In six experiments the flow of blood and the oxygen consumption of the various parts were determined while in the remaining seven, only the rate of blood flow was measured. In three of the studies, the animal was breathing oxygen throughout the course of the observations. The results in the one experiment in which morphine was

TABLE 1

Fractionation of the cardiac output and oxygen consumption

Experiment number	Weight of dog, kgm.	Cardiac output, cc. per minute		Flow of blood, cc. per minute					Cardiac output immediately after, cc. per minute	Oxygen consumption, cc. per minute					Oxygen consumption body, cc. per minute (spirometer)	Remarks
		Previous date	At beginning of experiment	Posterior part	Kidneys	Liver and portal	Upper extremities and head	Total		Posterior part	Kidneys	Liver and portal	Upper extremities and head	Total		
17	10.0	1,382	1,343	158	229	375	441	1,203	1,177	9.49	3.44	18.0	21.96	52.89	65.67	Breathing oxygen throughout
46	11.36	1,329	1,752	285	214	469	476	1,444	1,768	17.77	3.98	28.05	26.51	76.31	90.40	Breathing oxygen throughout
13	10.0	1,219	1,359	138	150	319	429	1,036	1,034	5.15	2.33	19.11	11.03	37.62	52.0	Breathing oxygen throughout
42	10.0	1,181	1,380	179	250	429	300	1,158	1,230	6.77	4.43	32.11	17.95	61.26	76.56	Breathing air
93	11.0	1,420	1,732	119	181	600	500	1,400	1,427	5.83	4.70	32.04	26.40	68.97	88.20	Breathing air
56	11.8	1,323	1,543	168	341	545	441	1,495	1,685	10.80	6.21	23.92	22.05	62.98	81.0	Breathing oxygen throughout Morphine 0.065 gm.
27	15.0	1,559		217	263	316	500	1,296								Breathing air
3	17.7	1,642		172	333	361	492	1,358								Breathing air
31	16.71	1,699		233	395	300	508	1,436								Breathing air
27B	14.8	1,559		217	263	316	500	1,296								Breathing air
27C	12.2	1,119		201	288	283	375	1,147								Breathing air
26	11.1	1,353		100	246	500	341	1,187								Breathing air
128	10.4	1,403		151	219	353	385	1,108								Breathing air
Ave.	12.5	1,399	1,518	180	259	397	438	1,274	1,387	9.30	4.18	25.54	20.98	60.00	75.64	

administered, were approximately the same as those obtained in the other studies.

Discussion. If one compares the average blood flow through the four parts in the thirteen experiments with the output of the heart as determined several days before the studies, it is found that the posterior part of the body contributed 12.9 per cent, the kidneys 18.5, the portal system and liver 28.4 and the upper extremities and head 31.3, making 91.1 per cent of the total. In six instances, cardiac output determinations were made

at the beginning and end of the experiment proper. The flow through the four parts equalled 84.9 per cent of the initial cardiac output determination, 92.9 per cent of the subsequent one and 88.9 per cent of the average of the two. The main portions of the body which were not included in the fractionation of the blood flow were the heart and the area drained by the azygos vein. Harrison, Friedman and Resnik (1936), using a modified Morawitz cannula, found that the percentage of the cardiac output passing through the coronary vessels ranged from 2.9 to 12.2 per cent, the mean value being 5.7 per cent. No figures are available on the rate of blood flow through the azygos vein.

It was found that the oxygen consumption of the posterior part of the body equalled 12.3 per cent, the kidneys 5.5, the liver and portal system 33.8 and the upper extremities and head 27.7 per cent of the entire quantity, the total being 79.3 per cent. The utilization of oxygen was lowest in the kidneys and highest in the portal system. Harrison, Friedman and Resnik (1936) found that approximately 10 per cent of the total oxygen absorbed was used by the heart, the range of values being from 5 to 23.5 per cent. Again, we have no figures on the oxygen consumption of the parts drained by the azygos vein.

SUMMARY

The blood flow and oxygen consumption of 1, the posterior part of the body; 2, kidneys; 3, portal system including the liver, and 4, upper extremities and head have been determined in unanesthetized dogs. These findings have been compared with the cardiac output and the total oxygen consumption of the body.

REFERENCES

(1) Mason, M. F., A. Blalock and T. R. Harrison. J. Biol. Chem. (Proc.) **114**: lxiv, 1936.

(2) Blalock, A. and M. F. Mason. This Journal. In press.

(3) Harrison, T. R., B. Friedman and H. Resnik. Arch. Int. Med. **57**: 927, 1936.

THE EFFECT OF ARTIFICIAL RADIANT ENERGY ON THE TISSUE TEMPERATURE GRADIENT IN MEN OF DIFFERENT SKIN COLORS AND AFTER ARTIFICIAL PIGMENTATION

HENRY LAURENS AND PAUL C. FOSTER

From the Laboratory of Physiology, School of Medicine, Tulane University, New Orleans, La.

Received for publication October 10, 1936

The pigment in the skin of man, according to some workers, acts as a screen against luminous and infrared rays, serving to localize the heating effect at the surface, from which it may more readily be lost (1). The advantage enjoyed by dark-skinned persons over fairer ones in a hot climate in the way of greater tolerance to the rays of the sun is thus assumed to be demonstrated. The negro's skin heats sooner and to a greater extent than that of the white man, he therefore sweats more copiously and the sweat evaporates more quickly owing to the higher temperature of the skin. However, no careful and exhaustive studies have been made of the influence of pigment on heat regulation in fair and dark skinned persons and Miescher's (2) observations indicate that the pigmented skin of the negro is not very advantageous to him in this way.

Our method is based on the tissue temperature gradient recorded by hypodermic thermocouple needles. The details of the apparatus have been published (3). Eleven male subjects with various natural skin-pigment types were used. Included among them were one light-skinned Chinese, a dark-brown Japanese, and a deep chocolate-colored Negro. The others were all white, with skins varying from light blond to dark brunette. In each case, except the Negro, the right leg was pigmented by exposure to a carbon arc, starting with small doses of energy and increasing the duration and strength of the exposure as rapidly as tolerance was established. In some cases "Sunshine" carbons were used; in others "Therapeutic C" carbons. The subjects varied greatly in their ability to acquire pigment, some developing deeply browned skin, others only a faint tan.

Wide bands of energy were employed, varying as to wave length limits and maximal intensity. Very long infrared radiation was obtained by electrically heating an iron plate (15 cm. in diameter and 1 cm. thick) to a temperature of 400°C. More than 90 per cent of the energy was longer than 3.0μ. An electric heater heated to a yellow red color, gave somewhat

shorter infrared, about 30 per cent of the energy being shorter than 3.0μ and 2.2 per cent shorter than 1.4μ. Still shorter infrared (about 600mμ to 3.0μ) was obtained by filtering the energy from a 25 ampere carbon arc or from a 1500 watt incandescent lamp through a glass cell containing 5 mm. of 0.5 per cent iodine in carbon bisulphide. The 1500 watt tungsten lamp was used in a special reflector and, when unfiltered, emitted practically no ultraviolet, about 4 per cent visible, about 35 per cent shorter than 1.4μ, and nearly 90 per cent shorter than 4.2μ. The wave lengths longer than 4.2μ came from the heated glass and reflector. The wave length of maximal intensity was around 940mμ and more than half of the total output was at wave lengths shorter than 2.0μ. Visible energy plus much short

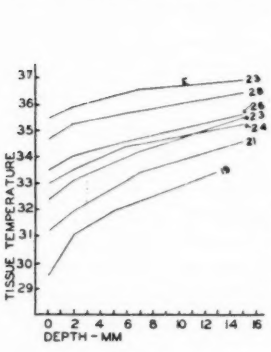


Fig. 1

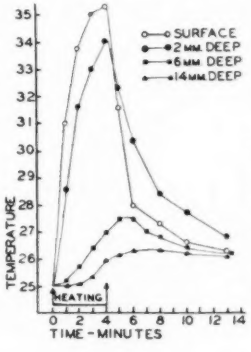


Fig. 2

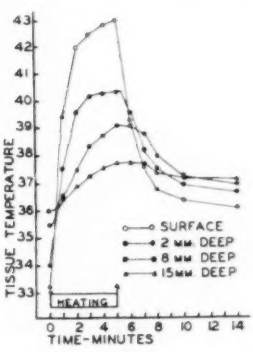


Fig. 3

Fig. 1. Tissue temperature gradients on anterior surface of thigh in the same subject at the various indicated room temperatures. Each curve represents a random choice from a series of determinations. Curve *E* was obtained during an intense ultraviolet erythema.

Fig. 2. Temperature rise at various depths in an agar jelly plate when the surface is heated with 1.0 g. cal. per sq. cm. per min. of long infrared.

Fig. 3. Temperature rise at various depths in the human thigh produced by heating the surface with 1.0 gm. cal. per sq. cm. per min. of long infrared.

infrared was obtained by filtering the radiation from a 25 ampere carbon arc or the 1500 watt incandescent lamp through a glass cell containing 5 mm. of water, also in a few cases by filtering such radiation through a heat-absorbing glass (Corning G392H). Glass filters are, however, unsatisfactory because they heat and become radiators of long infrared.

The subject reclined with both legs exposed to the air. Needles were inserted to depths 1, just subcutaneous; 2, between 5 and 8 mm., and 3, between 10 and 18 mm., and surface junctions put in place. Temperature equilibrium was reached in from 20 to 30 minutes after which the area was irradiated through a 15 x 15 cm. aperture in a screen. Temperature readings were made each minute during a period of irradiation, which was

continued until the temperatures at the various depths showed, practically, no further change. The cooling process was usually followed until the rate of temperature change was very slow. Several observations were made on one leg, and then the entire procedure repeated on the other, keeping conditions as nearly constant as possible. One hundred and thirty such sets of observations were made, using the various sources and dosages.

The initial tissue temperature gradient varied considerably from subject to subject, and also in the same subject under different conditions (4). The temperature of the surrounding air appeared to be the most important

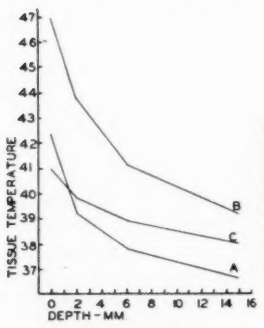


Fig. 4

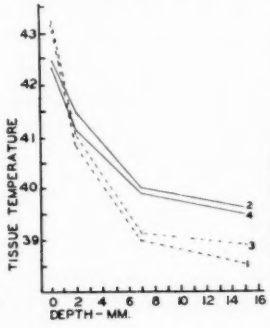


Fig. 5

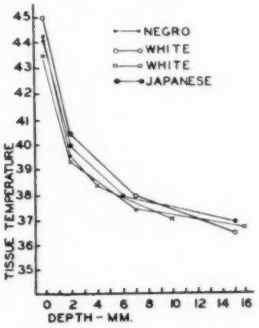


Fig. 6

Fig. 4. Effect of heating anterior surface of thigh with 1.0 g. cal. per sq. cm. per min. of long infrared; *A*, before occlusion of the circulation; *B*, during occlusion, and *C*, after occlusion. Each curve represents values obtained at the end of a five minute period of irradiation.

Fig. 5. Comparison of the effects of irradiating with 1.74 g. cal. per sq. cm. per min. from the electric heater (dotted line) with 1.79 g. cal. from a 1500 watt tungsten lamp (solid line). Each curve represents values at the end of a six minute period of exposure.

Fig. 6. The effect of 1.0 g. cal. per sq. cm. per min. doses of long infrared (electric heater) on a group of four different natural skin pigment types.

factor, and there is a fair correlation between this and the temperature gradient, which is on a higher level and less steep with increasing room temperatures (fig. 1). The amount of blood flowing through the skin is another important factor, shown strikingly when the temperature gradients are measured during an intense ultraviolet erythema (curve *E*, fig. 1).

There seems to be no correlation between the degree of natural pigmentation and the temperature gradient, but there is a tendency for the temperature to be higher on an artificially pigmented than on a non-pigmented leg. This may be due to the increased superficial blood flow following the irradiation (5).

The temperature response of skin and subcutaneous tissue to radiation is

quite similar to that seen when an artificial homogeneous medium like agar jelly is similarly irradiated (figs. 2 and 3). Qualitatively, blood flow constitutes the chief difference between them. The importance of this in temperature equalization was strikingly illustrated in several experiments in which skin was irradiated before, during and after occlusion of the circulation with a blood pressure cuff (fig. 4). During occlusion, *B*, the temperature at all levels increased excessively, and, when the blood was readmitted, *C*, the surface rise was less and the subsurface rise greater than before the circulation was occluded, *A*, due to reactive hyperemia, evidenced by extreme flushing, and by the fact that the temperature at all points on the occluded side was two or three degrees higher than corresponding points on the other side, even at the end of an hour. When long and intense exposures were used the surface and subsurface temperatures remained above the initial level for at least two hours.

Regardless of wave length or dose, the responses are qualitatively similar to those shown in figure 5. In the infrared bands the effects are very similar, the temperatures at all depths produced by a given amount of energy from the hot iron plate (long infrared) being practically identical with the temperatures produced by the same amount of energy from the electric heater (shorter infrared). The skin absorbs almost the same proportion of incident energy throughout the entire infrared range. Skin, both white and black, behaves as a black body for wave lengths longer than 2μ (6). A tungsten lamp, however, which is particularly rich in short infrared produced changes, quantitatively somewhat different. In figure 5 it will be noted that irradiation with the electric heater produced a greater rise of temperature at the surface than did the incandescent lamp, but that the deep temperatures were not so high, indicating a greater abundance of "penetrating rays" in the incandescent source. No radiant energy, however, penetrates through tissue to any considerable depth in any considerable amount. All but 5 per cent of even the most penetrating wave length (around 1.2μ) is absorbed at a depth of 2 mm., and all but 1 per cent at a depth of 3 mm. (6). However, a fair proportion of the energy does enter the skin and in dead skin 50 per cent of wave length 1.2μ penetrates to the level of the subcutaneous blood vessels (6). The blood in living skin causes a more rapid extinction. These facts, together with reflection, explain the differences in response to long infrared and to short infrared and luminous energy, as illustrated by figure 5. Long infrared is absorbed in the outermost layers of skin, and but little is reflected, since skin is essentially a black body to infrared longer than 2μ. A part of the heat is lost by radiation and evaporation, the remainder is conducted downward, or is carried by the blood to warm the deeper tissues. When an equal amount of energy from the 1500 watt tungsten lamp (a source rich in short infrared) falls on the skin surface, a greater proportion is reflected (since skin

does not behave as a black body to short infrared and visible), and a greater amount (maximal at 1.2μ) penetrates to some distance. These two factors combine to result in a smaller rise in surface temperature, but in higher subsurface temperatures, since the greater penetration heats the deeper layers in the vicinity of the blood vessels a little more.

Differences between long and short infrared sources are also seen when the time required for a rise in temperature at a depth of 2 mm. is observed. This is about 4 seconds on irradiation with 1.0 g. cal. per sq. cm. per min. with the 1500 watt incandescent lamp, and about 12 seconds with the electric heater. Regardless of the wavelength, heat is concentrated at the surface. Sub-surface temperatures were never found which exceeded those at the surface. This is not in accord with the widely quoted claim of Sonne and of Loewy and Dorno (1), that the luminous and short infrared rays are transmitted through skin to be absorbed in the region of the capillary network, resulting in temperatures 3.5 degrees higher at a depth of 5 mm., than at the surface.

There is marked similarity in the responses of the various subjects, although of different natural skin types, to varying amounts of energy in the different infrared spectral bands (fig. 6). The temperature changes in the different subjects were certainly no greater than those in the same subject on different days. The results with shorter wave bands were less satisfactory because of the difficulties encountered in obtaining sufficiently intense filtered energy. It appears certain, however, that natural skin pigment does little to alter the response to such radiation. The only noteworthy observation was that the responses in the Negro to equal doses emitted by the electric heater and by the 1500 watt lamp were closer than in the white subjects. We cannot yet imitate the spectral distribution of natural sunshine with any artificial source. Approximately 58 per cent of the energy emitted by the 1500 watt tungsten lamp is shorter than 3.0μ, whereas in solar radiation, as it reaches the earth, 99 per cent is shorter than 3.0μ. The sun has a larger proportion of "penetrating" rays than any artificial source and the final judgment relative to the protective action of pigment must be reserved until further work has been done with natural sunlight.

Again, when artificially pigmented skin is compared with non-pigmented skin of the same individual the results are similar. None of our records suggest that artificial pigment concentrates heat at the surface. In some subjects there was a tendency for the surface temperatures not to go so high and for the deeper temperatures to go a little higher on the artificially pigmented than on the normal side. These results are explicable on the basis of increased blood flow, but not on pigmentation *per se*.

Therapeutically, one spectral band possesses little advantage over another for heating deep tissues. It is possible to heat deeper tissues

about as high with long waved infrared as with shorter rays, since heat must get to deep structures by conduction and by heated blood. The shorter waved sources possess one advantage in that they cause less discomfort, because the heat is not concentrated quite so much at the level of the free nerve endings. All the subjects experienced the sensation of uncomfortable heat with skin temperatures of about 42°C. and of intolerable heat at about 47°C. With minor exceptions 1.8 g. cal. per sq. cm. per min. was about the maximal tolerated dose of long waved infrared. The same amount of shorter waved energy from the incandescent source produced a sensation of comfortable heat, the surface temperature rising less, but the deeper temperatures a little more, than when the longer infrared fell on the surface.

A series of observations on the Penetrex Lamp[1] (7) showed that the energy emitted behaves similarly, in so far as penetration is concerned, to that of the 1500 watt lamp. The filter, as might be suspected, becomes quite hot and therefore secondarily emits much long infrared, thus failing to fulfill its function in screening out such rays.

SUMMARY

1. The effect of radiant energy on surface and subcutaneous temperatures is similar to but not identical with that seen in artificial homogeneous media, such as an agar jelly plate. The difference is due to the heat-distributing function of the blood.

2. The response of skin and subcutaneous tissue to long infrared is such as would be expected from consideration of the fact that skin acts as a black body to long infrared ($>2\mu$), and that such infrared is not appreciably transmitted.

3. The response to short infrared and luminous energy is such as would be expected from consideration of the fact that skin does not act as a black body to such radiation, and that an appreciable percentage of these shorter wave lengths penetrates far enough through the skin to be absorbed in the region of the blood vessels.

4. Temperature responses support the view that no wave length is to be considered penetrating, in the sense that any considerable amount of energy is transmitted to any considerable depth.

5. Natural pigment offers no protection to deeper tissues against the longer wave lengths of infrared, and but little protection is offered against shorter infrared and visible.

6. Artificially pigmented skin may sometimes show a diminished rise in surface temperature when irradiated. This is believed to be a function of increased blood flow rather than of pigment.

[1] Dr. R. S. Danforth sent us this lamp at our request and we are glad to record here our thanks for his kindness.

REFERENCES

(1) LAURENS, H. The physiological effects of radiant energy. The Chemical Catalog Co., New York, p. 122, 1933.
Photochemistry in medicine, in symposia on quantitative biology, Cold Spring Harbor, **3:** 283, 1935.
The Scientific Monthly **42:** 312, 1936.

(2) MIESCHER, G. Strahlentherapie **35:** 403, 1930; **45:** 201, 1932; 2nd Cong. Internat. de la Lumière, 210, 1932.

(3) FOSTER, P. C. J. Lab. Clin. Med. **22:** 68, 1936.

(4) BAZETT, H. C. AND B. MCGLONE. This Journal **82:** 415, 1927.

(5) SHEARD, C. Symposia on quantitative biology, Cold Spring Harbor, **3:** 313, 1935.

(6) HARDY, J. D. AND C. MUSCHENHEIM. J. Clin. Investigation **13:** 817, 1934; **15:** 1, 1936.

(7) DANFORTH, R. S. Proc. Soc. Exper. Biol. and Med. **27:** 283, 1930.

PRESSORECEPTOR[1] REFLEXES FROM THE CAROTID SINUS[2]

CLAUDE V. WINDER

From the Department of Physiology, University of Michigan, Ann Arbor

Received for publication August 24, 1936

This report is presented from the standpoint that despite the large amount of work on carotid sinus reflexes the picture is yet incomplete in details and in proper emphasis of its parts. The study consists in a correlation of vascular, cardiac and respiratory responses with changes in intrasinus pulsating perfusion pressure, in many dogs anesthetized with morphine and urethane (pentobarbital in three experiments), under differing experimental circumstances.

The vascularly isolated carotid segments were perfused through the common carotid arteries as inlets and external carotid or lingual arteries as outlets, by means of the arrangement diagrammed in figure I. The system was immersed in a circulating water bath with temperature held at 38°C. electrically. The sharpness, rate and amplitude of pulsations, and the mean perfusion pressure were controlled. The perfusing fluid was a heparinized mixture of defibrinated dog's blood and Locke's solution continuously equilibrated with gas ranging from one experiment to another between 4.46 per cent and 6.09 per cent carbon dioxide, and 70 per cent and 93 per cent oxygen. Mean femoral arterial pressure and heart rate, perfusion pressure (mercury manometers), respiration (spirometer of a rebreathing circuit), time, and signals were recorded.

Several hundred observations were made on responses to changing intrasinus pressure, in 31 experiments. The sinus segments were completely isolated in 13 of the experiments, as indicated by one or more of the following tests: by absence of back-flow into the isolated segments when emptied and clamped off; by absence of loss of fluid from the perfusing circuit when maintained at high pressure; or by post-mortem "color-mass" injection into the sinus segments under considerable pressure, and subsequent dissection. Since it is now generally conceded that wide changes in cerebral arterial pressure do not alter vasomotor or cardiac activity, it is inconceivable that the remaining experiments were invalidated by the very small connections remaining between the perfused sinuses and the general circulation. In 6 of the 13 experiments in which

[1] Koch, 1931.

[2] This study was supported in part by a grant from the Rockefeller Foundation.

the sinus segments appeared perfectly isolated the systemic arterial pressure was 120 mm. Hg or over, indicating animals in very good experimental condition. Results of these 6 experiments will be stressed as typical, especially in considering vasomotor responses; but no statement not supported or qualified by the entire group of experiments will be made.

The vasomotor reflex. The following points were confirmed: 1. The vasomotor reflex arising from the carotid sinus is a very constant and definite entity. It is by far more striking and refractory to unfavorable

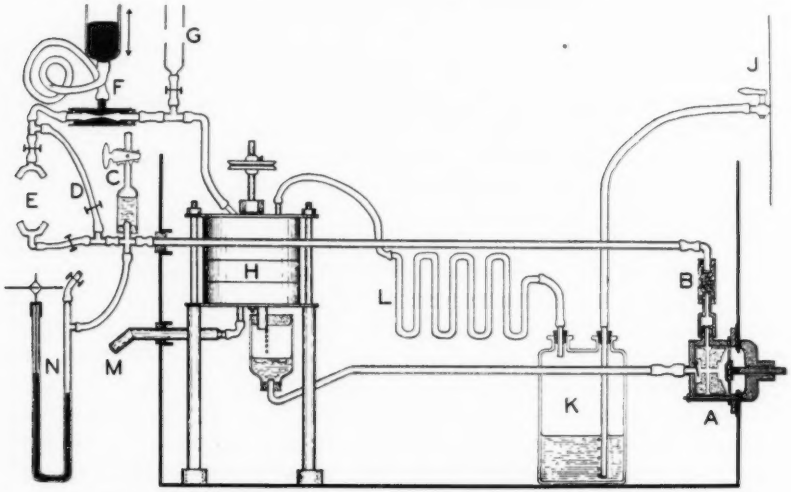


Fig. I. Diagram of continuous perfusion circuit. *A*, a modification of Hemingway's (1933) perfusion pump; *B*, glasswool filter; *C*, adjustable air-trap and cushion; *D*, by-pass; *E*, position of perfused carotid segments; *F*, artificial arterial resistance; *G*, large burette for administering fluids to the circuit; *H*, a four-segment motor-driven aerator of the type designed by Bayliss, Fee and Ogden (1928), supplied with a reservoir from which the pump draws; *J*, gas supply for continuous equilibration with perfusing fluid; *K*, Wolff bottle for saturating gas with water vapor; *L*, copper coil for control of gas temperature; *M*, gas outlet; *N*, manometer for recording mean perfusion pressure.

experimental conditions than either the cardiac or respiratory reflex (Hering, 1927; Koch, 1931). 2. It operates with striking fineness in response to ordinary fluctuations in pressure (fig. II, 2. See also Heymans, Bouckaert and Regniers, 1933, and Tournade, 1930). 3. Cutting the vagosympathetic-depressor trunks allows the sinuses great freedom in influencing systemic blood pressure (fig. II, 9. See Hering, 1927). Maximal, adequate, bilateral stimulation of the sinuses is capable of causing maximal or nearly maximal vascular relaxation. (Cf. Koch, 1931; Lim and Hsu, 1931.)

A correlation of blood pressure responses with types and degrees of stimulus changes and experimental circumstances, has led to additional information which has not been specifically considered before: When the pressure change in the sinuses is *unnaturally abrupt*, there is not merely a lag in attainment of the corresponding blood-pressure level but also a *secondary falling-back of the blood pressure change* to a more moderate, maintained level than that initially attained (fig. II, 3A, 4A, 4B, 7B, 7C, 8A; fig. III, 4, 5). Oscillations may or may not follow (fig. II, 5). For abrupt *increases* in intrasinus pressure this escape of the response is the usual thing in good preparations. It is not so commonly seen nor so pronounced in cases of abrupt *decreases* in intrasinus pressure. For less extensive or abrupt changes in stimulus level, or less reactive preparations, the escape may be absent (fig. II, 3B, 6).

"Vagotomy" reduces and retards the escape (fig. II, 8). The question arises as to how much of the escape component removed by vagotomy is attributable to the antagonistic action of the aortic afferent system, and how much to the vagal efferent path of a possibly poorly maintained sinus heart reflex. It is seen from the figures that before vagotomy the cardiac slowing or acceleration which enters into the blood-pressure change is poorly maintained. But Koch (1931) and Schneyer (1935) have shown that it is well maintained when the antagonistic aortic fibers are cut (vagus fibers left intact). It follows that the efferent path of an inherently poorly maintained sinus heart reflex is not involved. That is, that component of the escape removable by vagotomy must be due almost entirely to the correcting influence on both vasomotor and cardiac activity of the aortic nerves, instituted by the change in aortic pressure secondary to the change in sinus stimulus level. Any possible influence of the Bainbridge venogenic reflex cannot be evaluated because of a lack of definite information on venous pressure changes in these experiments.

But a considerable though less abrupt escape persists after vagotomy (fig. II, 8C, 9B, 10, 11, 12, 13, 15). This component, like the whole, is far more common and pronounced on abrupt increases in stimulus level than on decreases (fig. II, 9B, 10). In the case of the former one might speculate upon the possible rôle of initial adaptation of sinus receptors recorded electrically by Bronk and Stella (1935) using *non-pulsatile* perfusing pressures. A difference in effective central summation of a more continuous discharge during the abrupt rise in sinus pressure *with pulsations*, and of a less continuous discharge on attainment of a steady pulsatile level, would have to be considered. A possible effect of pulsations in the stimulating pressure on the duration of the adaptation may also be pertinent. In connection with the escape of the blood-pressure *rise* on *lowering* intrasinus pressure abruptly after vagotomy, one recalls a silent period in sinus receptor discharge of about 18 seconds' duration, observed

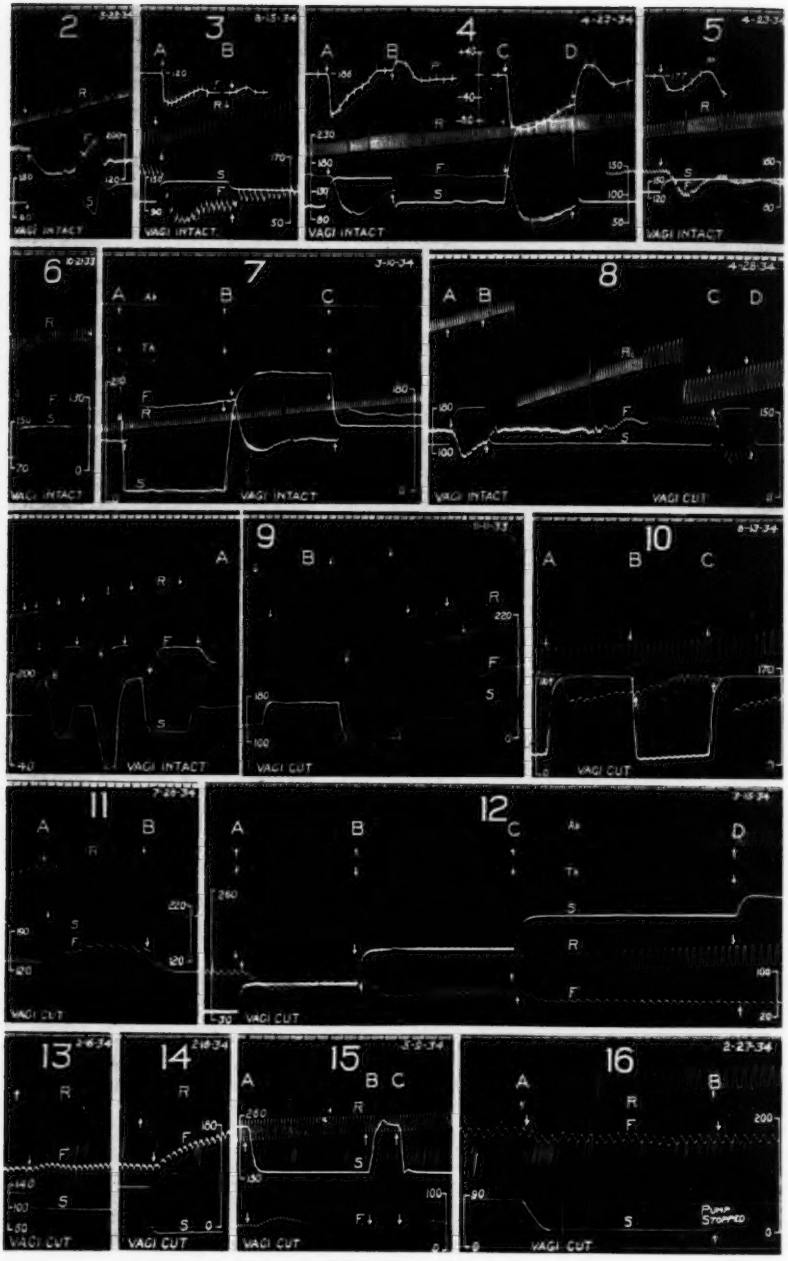

2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
VAGI INTACT
VAGI CUT
PUMP STOPPED

Fig. 11

by Bronk and Stella (1935) on lowering intrasinus pressure. In figure II, 15, for instance, when the mean sinus pressure is dropped from 240 to 160 mm. Hg, there is a rise in blood pressure which is ended in about 16 seconds by the beginning of a fall. It may be that the burst of vasomotor activity is ended by cessation of a relatively silent period in sinus impulses. Admittedly, there may be central causes of the postvagotomy escape components as important or more so than such peripheral ones. For instance, "rebound" from central inhibition must be considered.

Before vagotomy the escape is least (compare fig. II, 4A with 4C, and 7A with 7C), and after vagotomy it is absent (fig. II, 12C, 12D, 14, 15), when the intrasinus stimulating pressure is shifted to either maximal or subliminal; that is, either 200 to 250 or 40 to 60 mm. Hg (Koch, 1931; Lim and Hsu, 1931; Schneyer, 1934, 1935). It becomes obvious that when the stimulus is changed to either of these extremes, the escape seen in the *intact* animal is due almost if not quite entirely to correction by the aortic (and Bainbridge?) reflexes. Furthermore, in the case of the upper extreme, since as the aortic pressure falls cardio-aortic impulses are merely reduced to a minimum (Rijlant, 1932; Karasek, 1933), leaving the vasomotor mechanism to the unyielding influence of sinus impulses, the escape of the blood-pressure fall must be related to non-maintenance of cardiac inhibition alone (antagonistic reflexes). Thus the *absolute level* of the blood pressure (to be distinguished from the *amount* of *fall*) attained on maximal stimulation is lower before vagotomy than after by the hemodynamic effect of the cardiac inhibition, and can remain lower only so long as the latter is maintained (fig. II, 4C; fig. III, 2). For a change of stimulus in either direction to any *sub*maximal or *super*liminal level, the greater escape observed is due partly to these antagonizing reflexes, and partly to something else, no doubt including the characteristics of sinus receptor discharge considered above.

Then there must be reasons for non-function of the latter in cases of shifts of the stimulus to either extreme. In the case of a drop in stimulus to *sub*liminal, apparently one needs only recall that there is permanent cessation of impulses from the sinus receptors, as contrasted with a temporary silent period in the case of a fall of stimulus to any *super*liminal level. For the other extreme, if it be true that maximal bilateral sinus

Fig. II. Records showing vascular and respiratory pressoreceptor reflexes. *P*, plotted pulse rate per minute; *R*, spirometer record of respiration (inspiration on upstroke); *Ab*, abdominal movements; *Th*, thoracic movements; *F*, femoral arterial pressure; *S*, intrasinus pressure. At left of records, scales of perfusion pressures; at right, of arterial pressures. At uppermost level of each record, tracing of time in seconds. Record 16 shows reversal of the vascular reflex at low intrasinus pressures, believed by Schneyer (1934) to be due to a constant tug on the carotids.

stimulation is capable of causing maximal vasomotor relaxation, the absence of escape on such stimulation, after vagotomy, is readily understood; following whatever adaptation of receptors or change in rhythm of discharge that may initially take place when maximal stimulation is attained, impulses impinging centrally must still be sufficient to maintain maximal vascular relaxation.

The cardiac reflex. It is generally agreed that impulses from carotid sinus receptors normally cause inhibition of cardiac activity. Certain

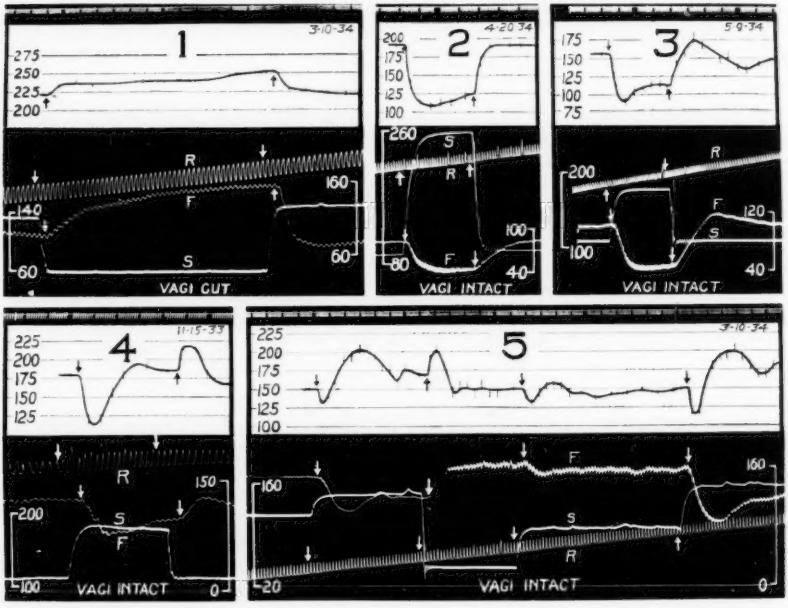


Fig. III. Records showing vascular, cardiac and respiratory pressoreceptor reflexes. Pulse rate per minute plotted on white backgrounds. Otherwise explanation same as for figure II.

of Hering's original observations, viz., that the heart reflex is of more sudden onset than the vasomotor reflex and attains its maximum usually within a very few seconds; that its magnitude (in the intact animal) is more variable than that of the vasomotor reflex; and that it is much more poorly maintained, are confirmed in these experiments (fig. II, 3–5, and fig. III, 2–5).

Since these results were recorded (1933–1934), Schneyer (1935) adequately analyzed the poor maintenance of the heart reflex in intact animals, concluding that the escape is due essentially to the correcting influence of the aortic afferent system, responding to the changing aortic

pressure (see also Spychala, 1933). It was observed in the present experiments that in those cases where the reflex was best maintained (fig. III, 2) the resting systemic blood pressure was low so that presumably a subnormal sensibility range of the aortic reflex zone was operating, and that the type of sinus stimulus shift was an *increase* to *maximal*. The more common characteristic was a more extensive partial escape (fig. III, 3) or a complete escape of the reflex (fig. III, 4).

The experiments agree further with Schneyer (1935) that with aortic nerves intact the reflex may be completely reversed. That is, on raising the intrasinus pressure the primary bradycardia may be violently converted to a tachycardia (fig. III, 5); and conversely, on lowering the intrasinus pressure the primary tachycardia may be changed secondarily to a bradycardia (fig. III, 5). This phenomenon was observed consistently in a good proportion of the experiments. A significant difference in method of these experiments and those of Schneyer, which in view of the similarity in results is the more important, is that the latter used a non-pulsatile intrasinus pressure with Moisejeff's (1927) "Blindsack" preparation, whereas the former used a more normal pulsating and flowing intrasinus pressure.

The far greater variability in magnitude and in degree of escape of the heart reflex than of the vasomotor reflex, and especially the tendency of the heart reflex to be completely reversed under the influence of intact aortic nerves, implies a considerably greater functional strength of the aortic system in cardiac regulation than in vasomotor regulation, and a greater strength of the aortic system than of the sinuses in cardiac regulation (cf. Koch, 1931; Izquierdo, 1930; Schneyer, 1935).

With the exception of Danielopolu and co-workers there is general agreement that carotid sinus reflex cardiac inhibition is brought about mainly through increased vagal activity and to a much less extent through decreased sympathetic activity, although Inaba (1931) could claim no constancy for the sympathetic efferent component. Danielopolu et al. believe that sinus impulses excite both sympathetic and parasympathetic activities in a parallel manner, the effect of one antagonizing that of the other, but the latter normally predominating. They term this concept "amphotropism" (Danielopolu, Marcu, Proca and Aslan, 1932) and use it as the basis for far-reaching prognostic and diagnostic principles (Danielopolu, 1929, 1930a, 1930b, 1936; Danielopolu, Marcu and Proca, 1932).

However, in every experiment of the present series in which heart rate was counted after vagotomy, moderately strong changes in intrasinus pressure caused unmistakable changes in heart rate, always in the same direction as those before vagotomy, thus demonstrating a reciprocal activity of sympathetic and parasympathetic cardiac innervation in response to changing sinus impulses (fig. III, 1). Although the changes were

much less than those seen (before escape) prior to vagotomy in satisfactory preparations, they amounted in some cases of strong stimulus change to more than 20 per cent of the resting rate. In general they were of much slower onset than with the vagus nerves functioning, which is in accord with the slower onset and recession of the sympathetic cardiac effect of direct efferent nerve stimulation. Frequently the change occurred in two phases (fig. III, 1). In such cases the first phase was usually completed in 10 to 50 seconds and the second, more gradual, began some time later. The second phase is very suggestive of a possible rôle of the reflex changes in adrenalin secretion arising from the sinus, reported from various laboratories (Heymans, Bouckaert and Regniers, 1933; Tournade, 1930; Aomura, 1930; Hartwich and Hessel, 1931; von Euler and Liljestrand, 1934; Heymans and Bouckaert, 1934; Nowak, 1934), or of a sympathin action.

In view of the constancy of the sympathetic (and humoral?) efferent component of the heart reflex in these experiments where an adequate stimulus was used, the unconstant findings of Inaba are probably attributable to the use of artificial electrical stimulation of a mixed afferent nerve supplying not only the sinus proper but also the carotid gland.

Our experiments add to the accumulating evidence that sympathetic and parasympathetic tonic activities respond to changing sinus impulses in a reciprocal manner with synergic effects on the organism. At the same time they emphasize the necessity of considering the functional state of the aortic nerves when studying sinus function. Antagonism by the former may not only partially or completely mask expected effects from independent sinus stimulation, but may even lead to confusion concerning the direction of sinus effects. Analysis of papers of Danielopolu et al. indicates that their results are understandable on the basis of experimental methods used, important among which was frequent disregard for the state of the aortic nerves. The latter seems particularly true for their results on dogs.

The respiratory reflex. Under the conditions of these experiments (urethane anesthesia), the respiratory reflex was the least constant of the three being considered. Frequently, intrasinus pressure changes which caused marked vascular responses had no obvious effect on respiration either before or after "vagotomy" (fig. II, 2, 6, 8, 12A; fig. III, 3, 5). Commonly, before vagotomy an increase in sinus stimulation was associated with increased respiration, rather than decreased as ordinarily reported (fig. II, 4, 5; fig. III, 4). In certain of such cases initial transient inhibitions were seen. After vagotomy this "reversal" of the response was not seen except in some cases where the systemic blood pressure fell to very low levels (fig. II, 12C, 15), or when the intrasinus pressure was brought to extreme heights. The reversal before vagotomy appears to be asso-

ciated with activity of the aortic afferent component of the vagus trunk, as is the reversal of the heart reflex.

For ordinary levels of intrasinus pressure, after vagotomy, a moderate increase in pressure usually causes more or less inhibition of respiration, and a decrease more or less release from the inhibition (fig. II, 9A, 10, 11, 12B, 13, 14).

SUMMARY

A continuous artificial circuit with continuous gaseous equilibration of the perfusing fluid has been used in studying carotid sinus reflexes in the dog anesthetized with morphine and urethane.

The vascular reflex was found to be a very constant and definite entity. Its response to ordinary fluctuations in pressure was of a striking fineness. Its maximal stimulation appeared capable of causing maximal and maintained vascular relaxation in the animal. In the absence of the aortic nerves complete removal of sinus stimulation in acute experiments appeared to completely unbridle vasomotor activity. A falling-back, or escape, of the initially attained blood-pressure level in response to *unnaturally abrupt* changes in sinus stimulation demonstrated the vicarious activity of the aortic nerves, and also appears amenable to correlation with characteristics of sinus receptor discharge observed by Bronk and Stella.

In animals with intact aortic nerves the carotid sinus heart reflex was considerably more variable in magnitude and much more poorly maintained than the vasomotor reflex (cf. Hering, 1927). In many cases the aortic afferent system was relatively so much more important in the regulation of heart rate than the carotid sinuses, that an effect initiated by the sinuses was secondarily reversed by the action of changing aortic pressure on aortic endings (cf. Schneyer, 1935). The sympathetic (and humoral?) efferent component of the sinus heart reflex was consistently demonstrable after "vagotomy." The concept of "amphotropism" of carotid sinus reflexes, as proposed by Danielopolu, is considered untenable.

The carotid sinus respiratory reflex was found to be the least constant of the three. Like the heart reflex, it was often completely reversed under the influence of intact aortic nerves.

REFERENCES

AOMURA, T. Tohoku J. Exper. Med. **15**: 1, 1930.

BAYLISS, L. E., A. R. FEE AND E. OGDEN. J. Physiol. **66**: 443, 1928.

BRONK, D. W. AND G. STELLA. This Journal **110**: 708, 1935.

DANIELOPOLU, D. Wien. Arch. f. inn. Med. **17**: 381, 1929; Ibid. **20**: 1, 1930a; Ibid. **20**: 157, 1930b; Présse méd. **44**: 497, 1936.

DANIELOPOLU, D., I. MARCU AND G. G. PROCA. Compt. rend. Soc. de biol. **109**: 109, 1932.

DANIELOPOLU, D., I. MARCU, G. G. PROCA AND A. ASLAN. Présse méd. **40**: 489, 1932.

EULER, U. S. VON AND G. LILJESTRAND. Skandinav. Arch. f. Physiol. **71**: 73, 1934.

HARTWICH, A. AND G. HESSEL. Ztschr. f. d. ges. exper. Med. **76**: 263, 1931.
HEMINGWAY, A. J. Physiol. **77**: 14P, 1933.
HERING, H. E. Die Karotissinusreflexe auf Herz und Gefässe. Dresden und Leipzig, 1927.
HEYMANS, C. AND J. J. BOUCKAERT. Arch. internat. de pharmacodyn. et de thérap. **48**: 191, 1934.
HEYMANS, C., J. J. BOUCKAERT AND P. REGNIERS. Le Sinus Carotidien et la Zone Homologue Cardio-Aortique. Paris, 1933.
INABA, T. Tohoku J. Exper. Med. **18**: 185, 1931.
IZQUIERDO, J. J. J. Physiol. **70**: 221, 1930.
KARASEK, F. Ann. de physiol. **9**: 793, 1933.
KOCH, E. Die reflektorische Selbsteuerung des Kreislaufes. Dresden und Leipzig, 1931.
LIM, R. K. S. AND F. Y. HSU. Chinese J. Physiol. **5**: 29, 1931.
MOISSEJEFF, E. Ztschr. f. d. ges. exper. Med. **53**: 696, 1927.
NOWAK, S. J. G. Compt. rend. Soc. de biol. **115**: 1731, 1934.
RIJLANT, P. Compt. rend. Soc. de biol. **110**: 589, 1932.
SCHNEYER, K. Pflüger's Arch. **235**: 176, 1934; Ztschr. f. Kreislaufforsch. **27**: 217, 1935.
SPYCHALA, V. Ztschr. f. Kreislaufforsch. **25**: 730, 1933.
TOURNADE, A. Lyon méd. **146**: 97, 1930.

ON THE MECHANISM OF STIMULATION OF CAROTID GLAND CHEMORECEPTORS[1, 2]

"CLAUDE" V. WINDER

From the Department of Physiology, University of Michigan, Ann Arbor

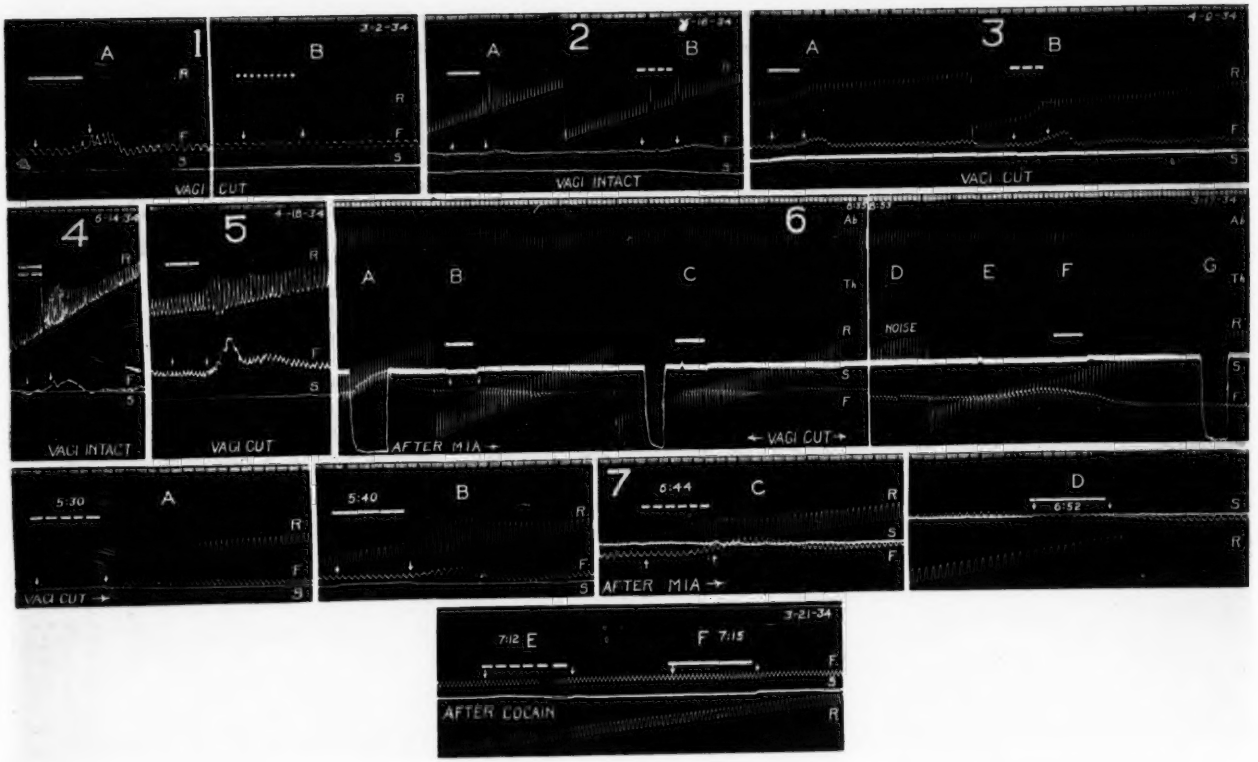
Received for publication August 24, 1936

It is now well established that chemoreceptors in the carotid gland are the point of departure of important anoxemic and hypercapnic, respiratory and circulatory reflexes; but little is known definitely of the fundamental mode of activation of these chemoreceptors by the various chemicals. An attempt is made here to contribute toward the solution of this problem.

The general method of attack was a comparison of reflex responses to lack of oxygen and excess carbon dioxide restricted to the carotid segments, before and after local poisoning with mono-iodoacetic acid, in dogs anesthetized with morphine and urethane. The carotid segments were perfused continuously by use of the arrangement outlined previously (Winder, 1937). It was necessary, in vascular isolation of the carotid segments, to preserve the minute arterial connections with the carotid glands. Accessory reservoirs for shunting anoxic, hypercapnic, asphyxial (anoxic and hypercapnic), or "control" fluid into the intake of the pump were necessary. After adjustment of the levels of the reservoirs, by means of valves the desired fluid could be shunted into the circuit and through the carotid segments at will, without change in perfusion pressure. To avoid reduction in gas pressure over the reservoir fluids, each was connected with a collapsible bag containing the proper gas. The fluid was a heparinized mixture of defibrinated dog's blood and Locke's solution. That used in the continuous circuit and in the accessory reservoirs was all taken from one mixture. The waterbath insured a common temperature for all. The gas used for equilibration of the continuous and control fluids ranged from 5.56 per cent to 6.09 per cent carbon dioxide, and from 74 per cent to over 93 per cent oxygen (dry). The anoxic fluid was equilibrated with gas of effectively the same carbon dioxide content (the difference ranged from 0.00 per cent to 0.20 per cent, averaging 0.05 per cent), and less than 0.5 per cent oxygen (except in one experiment in which the oxygen percentage was 1.15). The hypercapnic fluid was equilibrated

[1] A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Science, in the University of Michigan.

[2] This study was supported in part by a grant from the Rockefeller Foundation.



Figs. 1 to 7. Chemoreceptor reflexes before and after poisoning with MIA. For details see text. *R*, spirometer record of respiration (inspiration on upstroke); *Ab*, abdominal movements; *Th*, thoracic movements; *F*, femoral arterial pressure; *S*, intrasinus perfusion pressure. At the uppermost level of each record is a tracing of time in seconds. ———— indicates anoxic perfusing fluid; ———— indicates control perfusing fluid; ---------- indicates hypercapnic perfusing fluid.

with gas containing 35 per cent carbon dioxide and from 62 per cent to over 64 per cent oxygen (with two exceptions, when the concentrations were 25 per cent carbon dioxide and over 72 per cent oxygen, and 30 per cent carbon dioxide and over 69 per cent oxygen, respectively). The asphyxial fluid was equilibrated with gas containing 35 per cent carbon dioxide and less than 0.5 per cent oxygen. Such extremes of anoxia, hypercapnia and asphyxia were chosen deliberately for demonstrating whatever capability of response might remain after poisoning with mono-iodoacetic acid. The control fluid was abandoned after several experiments, because in no case did its flow through the carotid segments have any reflex effect on the organism. It seemed unquestionable that effects of the reservoir fluids would be due only to their gas pressures (see fig. 1, A and B).

In general the experimental sequence was as follows: Responses to the reservoir fluids were recorded before, or before and after vagotomy. The iodoacetic acid, free or neutralized with sodium carbonate solution, was slowly injected into the circuit. The pressoreceptor reflexes were occasionally tried as in the preceding paper. After an interval, responses to the reservoir fluids were again determined.

It has for some years been an accepted theory that in the chemical control of breathing the actions of anoxemia and hypercapnia may partially converge to the common factor of acidity *within* the controlling mechanism (Gesell, 1925, 1929). The fact that the actions of these regulants diverge in other respects (Gesell and Moyer, 1935a, 1935b) is not excluded by this concept, and the established importance of the carotid gland in the chemical regulation of breathing need not materially alter the theory, inasmuch as one needs only consider a part of the chemosensory mechanism located outside the medulla. Accordingly, we arrive at an accessible though minute sensory organ from which we may hope to learn something directly concerning the mode of stimulation by anoxemia and carbon dioxide.

Since Lundsgaard's (1930a, 1930b, 1930c, 1930d) original discovery that mono-iodoacetic acid (MIA) suppresses anaerobic glycolysis in frog and mammalian muscle and in yeast this poison has been accepted as a universal suppressor of glycolysis. Under certain conditions and for a certain time, concentrations of the poison just sufficient to prevent glycolysis seem not to affect cell "respiration", or enzymatic processes other than glycolysis, to any great extent. Such indications of the potential specificity of MIA in suppressing glycolysis, together with the fact that in spite of suppression of lactic acid formation a tissue may continue for some time to perform its characteristic work, drawing upon some more primary source of energy than glycolysis, point to its possible use in a crucial

test of the hypothesized rôle of glycolysis in anoxemic stimulation of sensory elements.

Henderson and Greenberg (1934) attempted such a test, but injected the poison into the intact animal rather than localizing its action, so that they were forced to use a dose (33 mgm. of MIA per kgm. body weight) inadequate for complete suppression of glycolysis (Lundsgaard, 1930a; Barrenscheen and Braun, 1931; Marenzi and Mundt, 1931; Field and Peeke, 1931; Neuss, 1931; Haldi, 1932; Haarman, 1932), in order to keep animals alive for experimentation. Indeed, before the anoxemic response was tested the animals had returned to an apparently normal state. The workers argued that glycolysis was at a standstill from the observation that during gradual oxygen deprivation there was no increase in *blood* lactic acid. But on considering their statement that there was no lactic acid increase in their unpoisoned control animals, this can scarcely serve as evidence to the end intended. Further, the modern acidity theory involves changes within cells, rather than blood, and recognizes a probable lack of parallelism. In fact, there are to be found indications in the literature that poisoned mammals die before lactic acid formation in brain or muscle, for instance, is completely suppressed (Neuss, 1931; Haarman, 1932), presumably due to blockage in the central nervous system (Ledebur, 1932). For such reasons, these workers' observation that anoxemic hyperpnea persisted in their "poisoned" animals cannot be considered as evidence that glycolysis is not involved in anoxemic hyperpnea.

In the present experiments, where the poison was limited to the carotid segments, any desired concentration could be applied to the receptor without endangering the remainder of the reflex mechanism. The volume of fluid in the continuous circuit was known to within 15 per cent, so that the poison concentration could be estimated that closely. Three groups of experiments were performed. Six experiments were done to ascertain whether anoxic stimulation at the carotid gland is affected in any way by concentrations of the poison that are reported to be adequate to suppress glycolysis in other tissues. The vagus trunks were severed. In the first, where the highest concentration of poison was used (0.0012 for 50 minutes, and 0.0025 for 16 minutes more, before the first dilution with blood from an accessory reservoir), the pressoreceptor and the anoxic reflexes were both abolished. In the second, where the lowest concentration was used (0.00007 plus or minus 0.00003, for 74 minutes before dilution), the pressoreceptor and anoxic reflexes both continued unaffected for 1½ hours after injection of MIA. In each of the remaining four experiments, however, where intermediate concentrations of poison were used (ranging from 0.00014 plus 0.00017 36 minutes later, with continuous dilution due to replenishing a constant fluid loss from the circuit, to 0.0007 undiluted for 20 minutes), the anoxic respiratory stimulation was entirely abolished

or so nearly so that any slight augmentation of ventilation was questionable, while the pressoreceptor reflexes persisted, though usually somewhat weakened.

If the anoxic reflex had been affected no more by the MIA than the pressoreceptor reflexes, it would have been fairly safe to conclude that glycolysis is not involved in the anoxic stimulation. The actual results of this first set of experiments, however, do not lead by themselves to a definite conclusion. The elimination of anoxic stimulation is definitely due to some action of the MIA, but one may justly ask whether it is specific elimination of glycolysis in the chemoreceptors or, for instance, surpassing the marginal concentration of MIA beyond which "respiration" of the chemoreceptors might be affected. A second series of eight experiments was therefore done, to ascertain whether the poison affects anoxic and hypercapnic responses differentially.

The poison concentrations in this series were of the same order of magnitude as those found in the first series to eliminate the normal anoxic response but not materially affect the pressoreceptor reflexes. The results of the first series were consistently confirmed in this respect. In one experiment, after poisoning neither anoxia nor hypercapnia elicited any response whatever, although the pressoreceptor reflexes had retained their original strength. In two others the anoxic and hypercapnic responses seemed abolished while the pressoreceptor reflexes were somewhat weakened. In both of these experiments increasing the perfusion pressure made it possible to elicit very slight and questionable responses with both anoxia and hypercapnia. In the remaining five experiments there were definite indications of a differential effect of the poison on anoxic and hypercapnic responses. At a time when the anoxic stimulation was entirely abolished or so very nearly so that its existence was questionable, the hypercapnic response remained, either apparently unaltered or more or less weakened. In two of these experiments the vascular isolations of the carotid segments were "complete", so that the persisting hypercapnic response could not have been an intracranial effect. In two of the three wherein small irregular branches had been left patent, external cocainization of the sinus segments completely abolished the persisting hypercapnic response, eliminating again the possibility of an intracranial effect. Records of the important stages in one experiment are shown in figure 7.

In such experiments it seems without further consideration justifiable to weight positive results much more heavily than negative, but there are circumstances suggesting the cause of the negative results of the first three experiments of this series. First, it is possible that the marginal concentration of MIA, beyond which its action becomes more generally toxic for the chemoreceptors, was surpassed. Second, certain observations indicate that the circulation through the gland was markedly retarded by

the poison; viz., an originally abrupt response to chemical stimulation of the gland commonly became more gradual and prolonged after the poisoning; and it was frequently necessary to increase the perfusion pressure to bring out a response. Work is available which bears on this retardation of circulation. Schmitt (1935) found intestinal smooth muscle to go into MIA contracture resembling that of striated muscle. In accord with this Neuss (1931) and Ueda (1934) report that MIA causes long-lasting and incompletely reversible constriction in frog and mammalian vascular beds. Dobrowolski (1933) and Irving (1934) report that MIA causes an increase in capillary permeability and edema. A contracture in the small arteries supplying the gland, coupled with edema, could cause a great disturbance in circulation and function. Such circumstances may account for considerable weakening or actual abolishment of the hypercapnic response along with the disappearance of the anoxic response, in a portion of experiments.

It seems safe to conclude from the positive results, then, that whereas the machinery of glycolysis is required for stimulation of the chemoreceptors by anoxia, it is not essential in the case of hypercapnia. We cannot go so far, however, as to claim that *no part* of the hypercapnic reaction involves glycolysis. To this point the experiments would not determine which of two possible consequences of glycolysis suppression eliminates the anoxic stimulation; viz., elimination of an actual link in the process of stimulation, or elimination of an energy source which would ordinarily be drawn upon under extreme anoxia. For that reason a third series of experiments was attempted, to determine whether hypercapnia can stimulate the poisoned chemoreceptors when they are simultaneously subjected to extreme anoxia. In addition to the anoxic and hypercapnic fluids, the "asphyxial" fluid was provided, equilibrated with the same pressure of carbon dioxide as the hypercapnic, and the same pressure of oxygen as the anoxic.

Unfortunately, in this series there were five out of seven experiments in which both the anoxic and hypercapnic, and also the asphyxic responses were abolished. In the remaining two experiments conditions were somewhat more, but not ideally, favorable for differential abolishment of the anoxic response. At stages of these two experiments where the normal anoxic stimulation was gone, the hypercapnic response persisted, though to be sure considerably weakened. More important, carbon dioxide was definitely able to stimulate under extreme anoxia (asphyxial fluid), which by itself was unable to stimulate. This is considered evidence that glycolysis is an actual link in setting up the local excitatory process by anoxia, rather than merely a necessary source of energy during the short period of anoxia. Work particularly from the laboratories of Embden and of Meyerhof indicates that MIA acts at a point in the glycolytic proc-

ess normally followed by acid intermediaries and finally lactic acid itself. It may not be premature to conclude, then, that anoxia appears to be dependent in some way upon processes of formation of acid products of glycolysis, for stimulation of the chemoreceptors of the carotid gland. It seems justifiable to assume here, as for the second series, that the lack of differential effect of MIA on anoxic and hypercapnic responses in so many experiments was due to actions of the poison other than specific suppression of glycolysis in the chemoreceptors. There seems no apparent reason, however, for the proportion of such experiments being higher than in the second series, unless it be that the MIA had undergone more decomposition.

In all three series of experiments there was usually a progressive increase in "resting" respiration beginning shortly after introduction of the MIA. The maximum increase was usually reached in about ½ hour. Such augmented resting breathing has been observed while the pressoreceptor reflexes appeared normal, the hypercapnic reflex persisted, and the anoxic response was abolished. Carotid gland excitability appeared increased for some stimulus, though abolished for anoxia. One recalls that hyperpnea is observed on intravenous injection of the poison into intact animals (Hall and Field, 1931; Neuss, 1931; Dobrowolski, 1933; Henderson and Greenberg, 1934). Hyperpnea from general poisoning is probably not entirely a result of carotid gland poisoning, but the latter must play at least a rôle.

In all three series, at that stage of an experiment commonly characterized by augmented resting ventilation, persisting pressoreceptor and hypercapnic reflexes, and abolished anoxic stimulation, there was a tendency for anoxia to cause delayed depression of ventilation (fig. 6, B and C) at a point which would have just followed the abolished normal stimulation. Since in two experiments where anoxic depression was marked there were small vessels remaining patent between the perfused carotid segments and the systemic circulation, while in one of those wherein the anoxic depression was least evident there were no indications of patent vessels (except the minute circulation into the carotid gland), the question arose as to whether the depression resulted from the anoxic fluid's reaching the medulla. There are circumstances to eliminate this possibility. After the records shown in figure 6, B and C, were taken, respiration gradually fell "spontaneously" to about the level existing during the depressions (fig. 6, E). At that stage anoxic depression almost completely failed (fig. 6, F). The slight swell is not significant, since the animal responded even more to noises in the room (fig. 6, D). If the anoxic depressions had been intracranial effects they would have persisted in about the same percental degree when the resting level of ventilation had changed. Two alternative explanations remain; viz., stimulation of *pressoreceptors* by

the anoxic fluid, or paralysis of *chemoreceptors* responding continuously to some such stimulant as the carbon dioxide pressure of the continuous fluid. Although the former could be ruled out for normal conditions (Bronk and Stella, 1935), it must be considered under MIA poisoning. It seems probable that the other alternative explains the depressions, since the level to which respiration finally fell when the carotid gland had apparently lost most of its irritability is about the same as that during the anoxic depressions (compare fig. 6, E, with B or C).

The question arises as to whether anoxemia and hypercapnia, with their respective acid mechanisms, actually stimulate a common set of receptors, or require two distinct types of receptors with functionally separate afferent fibers. It is remarkable that so far as they have been adequately studied, all irritants at the carotid gland cause the same general response pattern in the organism: hyperpnea, vasoconstriction and bradycardia. In the present experiments there was frequently a remarkable similarity in respiratory responses to anoxia and hypercapnia in the same animal, before and after vagotomy (fig. 2, A and B; 3, A and B). Cyanides and sulfides were avoided for fear of an irreversible effect on tissue oxidations that would have complicated observations with the iodoacetic acid procedure. But in those experiments wherein there seemed to be no great obstruction in the delicate carotid body circulation, short "blocks" of asphyxial fluid caused abrupt, intense respiratory responses which are scarcely distinguishable from those to cyanide or sulfide reported in other experiments (compare figs. 4 and 5 with cyanide or sulfide responses reproduced in other papers: Winder, Winder and Gesell, 1933; Winder and Winder, 1933; Winder, 1933). It was pointed out earlier (Winder and Winder, 1933) that cyanide and sulfide responses cannot be distinguished from one another. It is believed, then, that subserving the respiratory reflex there is either a common set of receptors and afferent fibers affected by various chemical conditions of the blood; or alternatively, two different sets with very similar central connections, normally subserving the anoxic and the hypercapnic reflexes, respectively. Bernthal (personal communication) has observed that the initial blood-flow changes in the dog's limb caused by various chemical changes at the carotid gland which are now known to increase the receptor discharge, are remarkably similar. Consequently, our belief concerning the respiratory reflex may be applied on the basis of Bernthal's work for the vasomotor reflex also. Further, Samaan and Stella (1935) observed only one type of impulse in the "sinus" nerve in response to chemical stimulation, whether anoxemia, hypercapnia, cyanide or nicotin was used. Zotterman (1935) pointed out that such impulses are propagated by small medullated fibers. These considerations suggest that if the respiratory, vasomotor, and it may be added, the cardiac reflexes, each responding to its various stimuli,

are not subserved by a single common set of fibers, the alternatively multiple sets must be of very similar type.

Even were respiratory responses to anoxia and hypercapnia at the carotid gland subserved by a common set of receptors, it would be surprising to find that the local excitatory process is set up in an identical manner by such differing conditions as deprivation of oxygen, overtension of carbon dioxide, and the many drugs and chemicals. In fact, Bernthal (personal communication), using prolonged exposures, has noted that the response to local anoxemia may build up gradually to a maximum whereas the response to local hypercapnia may reach its maximum sooner. The former reminds one of the gradual accumulation of lactic acid in blood and tissues (Gesell, Krueger, Gorham and Bernthal, 1930; Gesell, Krueger, Nicholson, Brassfield and Pelecovich, 1932), and the commonly observed gradual augmentation of breathing, during low alveolar oxygen administration.

SUMMARY

A study of anoxic, hypercapnic and asphyxic stimulation of the carotid gland before and after poisoning with mono-iodoacetic acid leads to the following observations: There is a progressive increase in ventilation of the animal during the onset of poisoning of the carotid segments, apparently due to an action at the carotid gland. Concentrations of the poison which abolish the reflex anoxic augmentation of ventilation or convert it to a late depression may affect the pressoreceptor reflexes very little. Under certain conditions the anoxic response may be thus abolished or converted while the hypercapnic response persists strongly. Hypercapnia appears able to stimulate the poisoned receptors under a degree of anoxia which by itself is incapable of stimulating.

It is concluded that anoxic stimulation of chemoreceptors of the carotid gland is dependent upon glycolytic processes, and that hypercapnic stimulation may be at least largely independent of such processes; that glycolysis is probably an actual link in setting up the local excitation by anoxia, rather than merely a necessary source of energy during the anoxia; and that the required part of that link is to be found in the formation of intermediary or final acid products of glycolysis.

The question is brought up concerning whether anoxemia and hypercapnia, each with its own acid mechanism, actually act upon a single set of receptors, or require two distinct sets.

REFERENCES

BARRENSCHEEN, H. K. AND K. BRAUN. Biochem. Ztschr. **231**: 144, 1931.
BRONK, D. W. AND G. STELLA. This Journal **110**: 708, 1935.
DOBROWOLSKI, N. Arch. internat. de pharmacodyn. et de thérap. **45**: 428, 1933.
FIELD, J. AND E. S. PEEKE. Proc. Soc. Exper. Biol. and Med. **29**: 358, 1931.

GESELL, R. Physiol. Rev. **5**: 551, 1925; Ergebn. d. Physiol. **28**: 346, 1929.
GESELL, R., H. KRUEGER, G. GORHAM AND T. BERNTHAL. This Journal **94**: 300, 1930.
GESELL, R., H. KRUEGER, H. NICHOLSON, C. BRASSFIELD AND M. PELECOVICH. This Journal **100**: 202, 1932.
GESELL, R. AND C. MOYER. Quart. J. Exper. Physiol. **24**: 331, 1935a; Ibid. **25**: 13, 1935b.
HAARMAN, W. Biochem. Ztschr. **256**: 326, 1932.
HALDI, J. This Journal **101**: 469, 1932.
HALL, V. E. AND J. FIELD. Proc. Soc. Exper. Biol. and Med. **29**: 360, 1931.
HENDERSON, Y. AND L. A. GREENBERG. This Journal **107**: 37, 1934.
IRVING, J. T. J. Physiol. **80**: 360, 1934.
LEDEBUR, J. Pflüger's Arch. **230**: 229, 1932.
LUNDSGAARD, E. Biochem. Ztschr. **217**: 162, 1930a; Ibid. **220**: 1, 1930b; Ibid. **220**: 8, 1930c; Ibid. **227**: 51, 1930d.
MARENZI, A. D. AND W. A. MUNDT. Compt. rend. Soc. de biol. **108**: 127, 1931.
NEUSS, F. Arch. f. exper. Path. u. Pharmakol. **160**: 551, 1931.
SAMAAN, A. AND G. STELLA. J. Physiol. **85**: 7P, 1935.
SCHMITT, E. Ztschr. f. Biol. **96**: 467, 1935.
UEDA, J. Jap. J. M. Sc., Tr., iv, Pharmacol. **8**: 45, 1934.
WINDER, C. V. This Journal **106**: 28, 1933.
Ibid. (preceding paper).
WINDER, C. V. AND H. O. WINDER. This Journal **105**: 337, 1933.
WINDER, C. V., H. O. WINDER AND R. GESELL. This Journal **105**: 311, 1933.
ZOTTERMAN, Y. Skandinav. Arch. f. Physiol. **72**: 73, 1935.

PRESSURE PULSES AND BLOOD PRESSURE VALUES IN UNANESTHETIZED DOGS[1]

DONALD E. GREGG, RICHARD W. ECKSTEIN AND MEYER H. FINEBERG

From the Department of Physiology, Western Reserve University Medical School, Cleveland, Ohio

Received for publication September 30, 1936

At present two views are held concerning the relationship between left ventricular and aortic pressure curves as regards contour and ordinate value. The one, sponsored by Wiggers (5) on the basis of experiments on anesthetized dogs with open chests and subscribed to by many others, holds that the systolic portions of both curves are parallel and of approximately the same height, whereas the other, most recently sponsored by Hamilton and associates (2, 3, 4), maintains on theoretical grounds and experimental work on unanesthetized dogs that during systole the apical intraventricular pressure must and does exceed the aortic to insure that blood shall flow from the ventricle to the aorta. Failure to obtain curves showing such pressure differences (indicating low or zero velocity in the ventricular apex and high velocity in the aorta) is ascribed to various factors which tend to reduce the kinetic factor and hence minimize the excess ventricular pressure; such as reduction of cardiac output through anesthesia and surgical shock and connection of the intraventricular manometer to the upper portion of the ventricle, a region in which the blood has nearly attained the aortic velocity.

PROCEDURE. To obtain an answer to the question, attention was directed first of all toward devising ways and means of determining accurately the pressure pulses from the heart and arterial system of normal well-trained dogs, and secondly, toward making the circulatory system more accessible so that we could be sure of the exact location of our cannulae.

For this purpose the Wiggers universal manometer cannot be used because attachment to it of the necessary, long, small-bore cannula results in an inefficient instrument of low frequency. The small, hypodermic manometer of Hamilton (2) overcomes these difficulties and represents a distinct contribution to the tools available for investigating circulatory

[1] The expenses of this investigation were defrayed by a grant from the Ella Sachs Plotz Foundation.

problems. In our work, the Hamilton type of manometer was used with various modifications as shown in figure 1. While the fundamental principle of the manometer (namely, a large effective mass combined with a very high volume-elasticity coefficient) has been retained, certain changes have been made which allow greater ease in mechanical manipulation and filling, greater sensitivity (larger curves) without sacrificing the manometer efficiency and which also give more uniform calibration curves.

The manometer proper consists of a thick walled brass cylinder 5 to 7 cm. long by 4 mm. internal diameter whose front end has been machined down so that after attachment of the membrane and mirror at *B* the manometer can be inserted into its carriage. A detachable lead tube, *D*, of varying length is attached to it by a threaded joint, *E*, and to the needle, *S*, by a tapered brass ring soldered to it so that it forms a sleeve joint with base, *R*, of the hypodermic needle. The making of the manometer in three sections has certain technical advantages. First, the manometer barrel can be easily filled by thrusting into it as far as its membrane, a brass tube or catheter, slightly smaller in diameter and connected to a sodium citrate bottle. The other portions of the manometer, after being jointed to the barrel, are then filled through stopcock *A*. Secondly, the sleeve connection at *R* allows removal and cleaning of an occluded needle without dismounting the manometer. Finally the manometer can be calibrated at various times during an experiment by shutting stopcock *O*, and opening stopcock *A*, which is connected to a sodium citrate bottle.

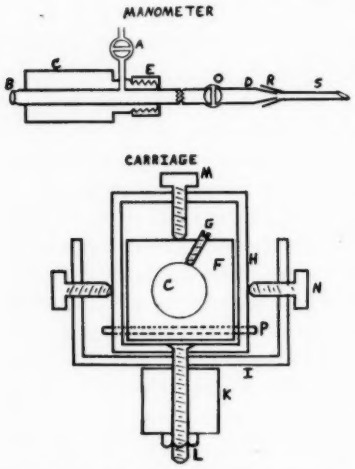


Fig. 1. Drawing of manometer and carriage used for recording blood pressures optically.

Although the telescope mounting of the manometer as used by Hamilton is satisfactory for some experiments, it lacks the flexibility and rapidity of adjustment that is so often required. Hence we have substituted the brass carriage shown in figure 1. The manometer, *C*, fits snugly into the solid brass block, *F*, and is held in place by set screws, *G*. This unit fits on its two lateral sides into a heavy brass casing, *H*, to which it is attached by a brass pin, *P*, thrust through the front end of *F* and *H*. This whole assembly is pulled snugly against *I* by means of the stout brass rod, *L*, (threaded at its lower end) and passing through brass block, *K*. Vertical

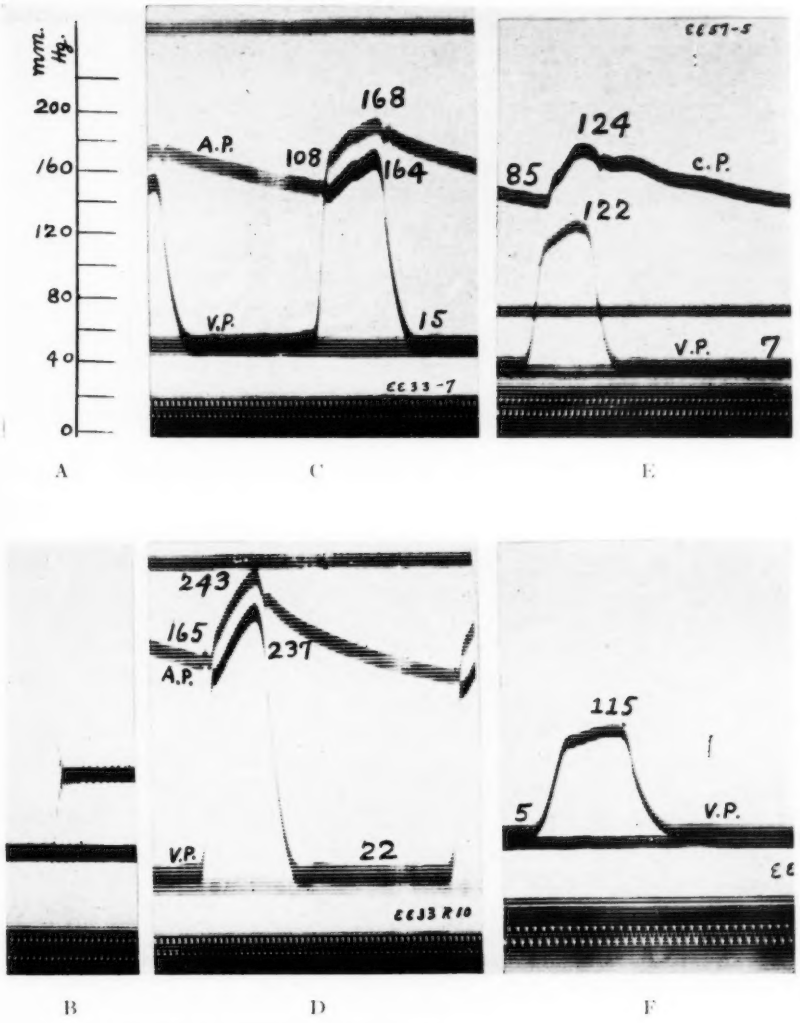


Fig. 2. Records of arterial and left ventricular pressures used for determining relative forms and pressure values under different dynamic conditions. A, typical calibration chart. B, frequency determination (135 per second). C and D, records taken before and after epinephrine injection in locally anesthetized dog. E, basal blood pressures from carotid loop and fixed ventricular apex. F, basal ventricular pressure in unoperated normal dog. In this and subsequent figures A.P. = aortic pressure; C.P. = carotid pressure; V.P. = left ventricular pressure; Time, 0.02 second.

movements of the manometer are obtained by adjusting screws, *M*, on the back portion of *H*. Lateral movements are obtained by two screws, *N*, attached to the non-movable section, *I*.

The aperture, *B*, upon which is placed the recording membrane utilizes the segment principle. For a membrane, shim brass and silver as advocated by Hamilton and associates and also heavy sheet rubber have been tried. However, the sensitivity with the metal membranes is so different at various pressure levels that they have been discarded and heavy rubber membranes tightly held in place by flexible wire have been substituted. With these membranes the calibration curves are more nearly uniform (fig. 2A) (except at quite low pressures where the sensitivity is somewhat greater) and remain unchanged for long periods of time. In addition sufficient sensitivity can be obtained at 5 meters' projection (without sacrificing the efficiency of the system) to permit registration of fairly large curves capable of rapid scrutiny, whereas the curves obtained with the metal membranes generally require micro-calibration and analysis.

In using the membranes, we have occasionally observed the phenomenon of hysteresis, especially when the pressure is suddenly lowered in the manometer system. All experiments are checked for its presence and those in which the membrane does not follow rapid pressure changes are discarded.

At times the base line mirror (not shown in fig. 1) is mounted directly on an immovable point of the manometer carriage but we prefer placing it on a hinge attached in front to the carriage so that it can be rotated vertically or horizontally and independently of the recording mirror.

The lighting system and mirrors are essentially those described by Hamilton except that we have used a series of adjustable slits in front of a projection bulb, the filaments of which are in line with the manometers rather than a single vertical slit with the projection bulb filaments parallel to the manometers.

The manometers as used had frequencies ranging from 130 to 300 per second (fig. 2B), the actual frequency depending on the membrane and bore and length of the attached hypodermic needle.

These manometers were used on several types of dog preparations. In the first experimental series, the procedure of Hamilton was duplicated. In morphinized dogs, the left ventricle and proximal carotid artery were entered (after surgical exposure of the artery under procaine anesthesia) by hypodermic needles (18–20 and 20–24 gauge respectively) and connected to the manometer just described. In a second experimental series the arterial pressure was obtained from the aorta by means of a long trocar pushed down the carotid to the aortic valves and joined to the recording manometer. In addition in both series, the possible modifications induced by general anesthesia, epinephrine, and by opening the chest on the

relationship of the left ventricular and arterial pressure curves were studied. The subsequent opening of the chest in many of these experiments enabled us to determine the exact location of the needle in the ventricle and to evaluate the possibility of cardiac tamponade following ventricular puncture.

However both types of experiments have the drawback that the position of the intraventricular needle cannot be determined unless the animal is sacrificed. Hence a third series of animals was prepared. Quiet dogs were selected from stock and trained to lie untied for hours at a time on soft beds provided for them. They were then operated on under ether anesthesia as follows, to make the circulatory system more accessible for puncture. For recording intraventricular pressures, a portion of the rib over the apex of the heart was resected, the pericardium slit and then the apex of the left ventricle and its surrounding pericardium were sutured in circular fashion to the bordering intercostal muscles so that the apex was anchored just under the skin. Thus as soon as the intraventricular needle had penetrated the skin, it was in contact with the apex of the heart and we could be reasonably certain of the exact portion of the ventricle penetrated. To insure that the pressure in all regions of the ventricle was recorded, a long needle was inserted in a variety of ways; directly through the apex and for varying distances toward or to the base, and also laterally through the apex with the needle tip directed toward the base or apex of the heart.

To obtain pressure curves from the arterial system, a Van Leersun loop was made of the left carotid and approximately three weeks allowed for operative recovery. On the experimental day the proximal artery was entered by a short, straight needle without anesthesia or dissection.[2] After withdrawal of the needle at the close of an experiment, the artery was gently compressed (2–3 min.) to avoid hemorrhage into the loop. These preparations, if not punctured too often, last a long time. Both arterial and ventricular punctures could be made repeatedly from day to day without any noticeable discomfort to the dog and without even a local anesthetic.

Results. In figure 2 C, D, are shown the changes that occur in the aortic and left ventricular pressure curves in a typical experiment in which the dog was maintained under different dynamic conditions. The dog, untrained and locally anesthetized, was from our second series in which the arterial pressure was taken from the aorta and the left ventricular pressure from the apical region by a hypodermic needle thrust through the lateral aspect of the ventricle. (The point at which the needle entered the

[2] We are greatly indebted to Doctors Beck and Mautz of the Department of Surgery for the cardiac operations and to Doctor Goldblatt of the Department of Pathology for preparation of the carotid loops.

ventricle was determined by sacrificing the animal at the end of the experiment.) The curves in figure 2 C were taken soon after the insertion of the hypodermic needle. The manometers differ slightly in sensitivity. The heart rate is 40 per minute although in most of the beats the rate was around 80, the variations being due to the normal sinus arrhythmia. During diastole the ventricular pressure is maintained at 15 mm. Hg and rises steeply during the ventricular isometric contraction period and then more gradually but still quite rapidly and smoothly throughout the duration of systole to reach a peak pressure of 164 mm. Hg. This is followed by a period of rapid pressure drop during the isometric relaxation period. The aortic curve parallels very closely that of the ventricle while the aortic valves are open with systolic and diastolic pressures of 168 and 108 mm. Hg. (The difference of 4 mm. Hg between the two systolic pressures is within the range of experimental error.)

If an excess of ventricular over aortic pressure is to be demonstrated, its presence should, in accordance with Hamilton's reasoning, be magnified by the injection of a substance such as epinephrine which increases the velocity of the blood leaving the heart. Accordingly epinephrine[3] was injected into a leg vein and the curves of D were taken. The systolic portions of the ventricular and aortic curves, although rising and falling more rapidly than in C, have essentially duplicate contours with an aortic pressure of 243/165 and a ventricular pressure of 237/22 mm. Hg. It is obvious that such curves showing agreement in contour and pressure values of their systolic portions differ from those reported by Hamilton. In order that the relative contours may be examined more in detail, the curves in figure 2 C, D, have been enlarged by projection and brought to identical scale in figure 3 A, B.

Such a relationship also holds regardless of the condition of the circulation. The curves in figure 3 C were taken later in the same experiment a few minutes after administration of sodium amytal anesthesia.[4] The heart rate increases to 136 per minute, the aortic pressure drops to 126/89 mm. Hg and the ventricular to 121/14 mm. Hg. During systole the pressure curves agree in contour but both present a rounded contour with the point of maximum pressure in mid-systole. After giving epinephrine, the curves in D were recorded. The heart rate decreases to 92 per minute. The relative contours and pressure values of the curves agree with those in A and B. In E the chest has been opened and epinephrine injected. The systolic portions of the aortic and ventricular curves have similar rounded contours with the maximum pressure late in systole.

[3] In all tracings shown in this paper epinephrine was given to accentuate any possible pressure difference between the left ventricle and aorta or carotid.

[4] We are indebted to the Eli Lilly Co. for furnishing a generous supply of sodium amytal.

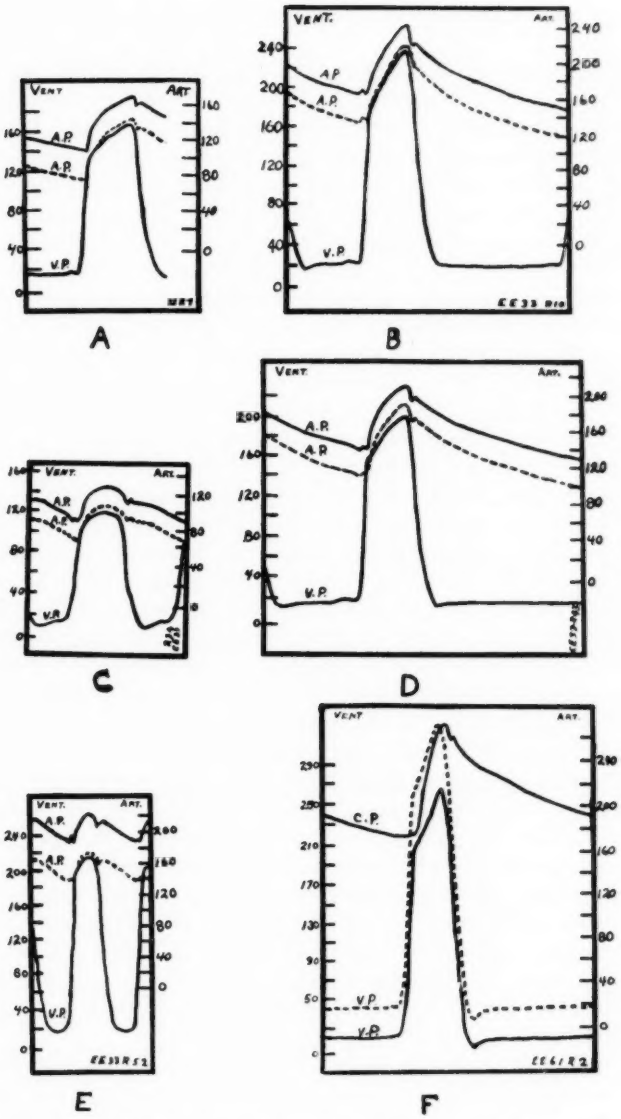


Fig. 3. Chart showing reconstructions from curves of figures 2, 4 (and also others) in which curves have been placed on same ordinate scale. Charts A, B = curves C, D of figure 2. Chart C = effect of general anesthesia. Charts D, E = effect of epinephrine after general anesthesia and opening chest respectively. Chart F = curve E of figure 4. Dotted line curve in A through E = aortic curve reconstructed to ventricular ordinate scale. Dotted curve in F = ventricular curve placed on aortic ordinate scale. Ordinates = millimeters of Hg.

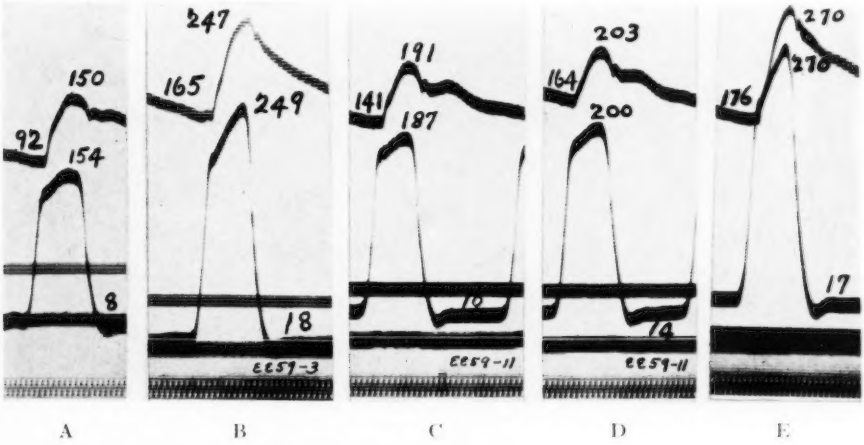
Such records definitely indicate that although the systolic and diastolic blood pressures and contours of the ventricular and aortic pressure curves are materially altered under different dynamic conditions, they always agree as to ordinate value and contour under any one condition.

To obviate the criticism that the ventricular needle (although in the lower third of the ventricular cavity) must be at the extreme apex to record the maximum ventricular pressure, curves were recorded from dogs of our third series in which the carotid pressure was taken from a Van Leersum loop with the needle opening directed toward the heart and ventricular pressure from the immobilized apex. The ventricular needle was inserted under the skin and while the apex was palpated, the needle was thrust into the apex and gradually withdrawn until sodium citrate under high pressure would scarcely flow through the needle. The needle was then advanced slightly further until the citrate level dropped rapidly. This was considered to be the apex of the heart.

As a preliminary step in most of these and subsequent experiments, blood pressure determinations were made under basal conditions. The dogs, carefully trained, were maintained under conditions such as obtain when a basal metabolic determination is made, that is, the animals were in a post-absorptive state and underwent a preliminary rest period of 15 to 30 minutes. Extraneous noises were excluded and finally no records were taken for some minutes after the insertion of needles or after a needle had been flushed with sodium citrate or heparinized Locke's solution. Rigid adherence to these conditions always results in blood pressures of the order of magnitude (or somewhat higher) of those shown in figure 2, E (124/85 mm. Hg) in which the carotid pressure was taken from a carotid loop and the left ventricular from the apex of the heart anchored to the chest wall. The ventricular curve in F approximates that in E and was taken from the lower portion of the left ventricular cavity of a well trained, basal dog under procaine anesthesia but in which the ventricle had not been sutured to the chest wall. This curve is added because it serves to emphasize that the operative procedures, which allowed us always to be sure of the exact location of the ventricular needle tip and which largely eliminated the possibility of puncturing a coronary vessel, did not embarrass the circulation or affect the pressure pulse.

Such pressures are considerably less than those reported by Goldblatt and co-workers (1) using the palpatory method on carotid loops and those of Hamilton (2) in supposedly basal dogs under morphine anesthesia. Our values are also much higher (168/108 mm. Hg) when obtained on morphinized dogs not well trained (fig. 2 C) or in which operative procedures are undertaken on the experimental day. Even with well trained dogs, systolic values of 140 to 170 mm. Hg are the rule unless the above precautionary measures are adopted.

After recording basal pressures the curves in figure 4 A, B, were taken with the above procedure before and after injection of epinephrine, the latter being given to magnify, if possible, any differences in aortic and ventricular pressures. As far as it is possible to judge of aortic pressure changes from curves recorded from the carotid, the contour and magnitude are essentially the same. That the apex and base of the heart also yield similar curves is evidenced in figure 4 C, D in which epinephrine had been given. In C the ventricular needle had been pushed through the apex so that its tip must have reached the base of the ventricular cavity. It was then withdrawn to the apex (as determined by the above test) and the curves of D recorded. When due allowance is made for the transmission



A B C D E

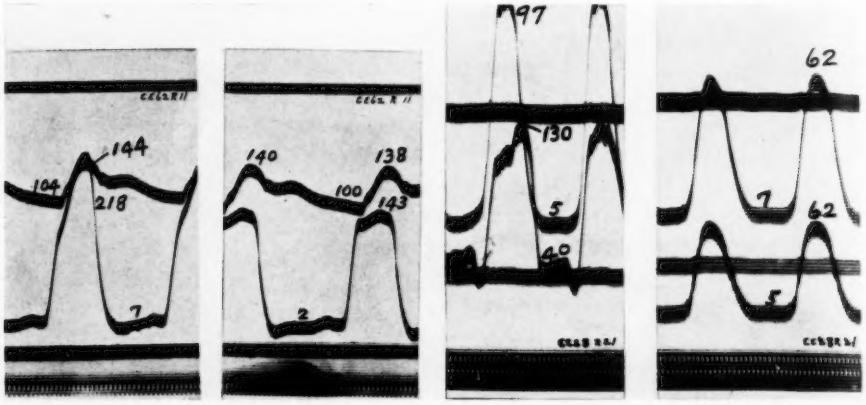
Fig. 4. Left ventricular and carotid pressure curves with the ventricular needle in various positions. A, ventricular needle at apex. B, ventricular needle at apex after epinephrine. C and D, ventricular cannulae at base and apex respectively after epinephrine. E, ventricular needle laterally through apex.

time of the carotid pulse, the curves and relative pressures in figure 4 are similar to those of figures 2 and 3.

It is remotely conceivable that, since the needle opening was not directed toward the apex, the full magnitude of the end pressure in the ventricle might not have been recorded especially when the needle tip was at the apex. Therefore as supplementary tests on the same operated dogs, the ventricular needle was inserted laterally through the apex and with its opening directed toward the tip of the heart. Such curves, after the administration of epinephrine, are reproduced in figure 4 E. Since the sensitivities of the ventricular and carotid manometers are quite different in the curves of figure 4, representative curves (fig. 4 E) have been enlarged

and placed on the same ordinate scale in figure 3 F to facilitate contour comparisons. The curves display no essential differences in relative forms and pressures from the others and need no further comment. The locus from which pressures are recorded and also the direction of the needle are immaterial in registering pressure pulses from the ventricle. We are therefore unable to repeat the results of Hamilton or to substantiate the dynamic theory of cardiac action evolved on its basis.

Discussion. Our records all indicate that there is no recordable region of the left ventricular chamber in which the systolic pressure is greater than any other. The question remains how curves such as those published



A B C D

Fig. 5. Curves showing abnormal relations of ventricular and carotid curves. A, apical ventricular curve with needle not in intraventricular cavity. B, normal apical ventricular curve. C and D, left ventricular pressure curves from the exposed heart. In C, upper curve taken from mid-portion of ventricular cavity; lower curve from intraventricular septum. D, control with both curves from left intraventricular chamber.

by Hamilton and also by others were obtained. We attempted to duplicate such curves by inserting the ventricular needle so that sodium citrate under pressure ran into the manometer and through the needle very slowly or not at all. Such impedance suggests that the point did not lie free in the cavity and most probably was either in, or tightly pressed against the myocardium or papillary muscles. Under such conditions curves such as those of figure 5 were obtained from our third series of operated dogs. In A, the ventricular needle had been inserted through the apex and then withdrawn until citrate flowed through it with great difficulty. In B, it was inserted somewhat further until sodium citrate easily ran into the

ventricle. In the first curves the ventricular pressure rises to a maximum of 218 mm. Hg compared with 144 mm. Hg in the carotid, which indicates that there is a region of the heart (probably not in the ventricular cavity) from which ventricular pressures in excess of the carotid can be obtained. The registration of such curves is not restricted to the apex, for similar records showing excess ventricular pressures have also been obtained from the same type of preparation when the ventricle was similarly entered midway between apex and base and probably through the intraventricular septum.

Simultaneous ventricular pressure tracings differing in contour and magnitude can also be obtained in open chest experiments. At the time the curves of figure 5 C, D, were recorded, the heart was in poor condition. The upper curves were recorded by a manometer connected to a needle inserted into the upper portion of the left ventricular cavity and the lower curves by a needle used to explore different regions of the left ventricular chamber and wall. The curves in C were taken after the exploring needle had been inserted into the ventricle through the intraventricular septum about midway between base and apex and then partially withdrawn so that citrate under pressure scarcely flowed. The systolic pressure recorded in the lower curve is 130 mm. Hg as compared with 97 mm. Hg by the needle known to be in the ventricular cavity. Record D was taken as a control after the exploring needle had been reinserted into the ventricular cavity.

Such experimental results indicate *a*, that it is possible to obtain higher pressures from certain regions of the left ventricle than from the aorta, carotid or from other ventricular regions; *b*, that such higher pressures are not restricted to the apex but are obtainable from various portions of the ventricle; *c*, that such a pressure difference is not a function of the dynamic condition of the circulation for it can be found with high and low blood pressures, pulse pressures and stroke volumes, in normal and unanesthetized dogs with or without the heart exposed.

Since such pressure differences have never been recorded when the ventricular needle tip is free in the cavity but only occur when its opening is occluded to the extent that fluid flows with great difficulty through it, we have reached the conclusion that this higher pressure is not existent in the lumen of the ventricle.

SUMMARY

1. The relative contours and pressure values in the left ventricle and aorta or carotid artery of the dog have been studied by means of a modified Hamilton manometer. The manometers were connected to several types of dog preparations, (well trained basal dogs, normal dogs under local or general anesthesia, and also trained basal dogs in which the apex of the

heart had been previously sutured to the chest wall and an exteriorized loop of a carotid made), maintained under different circulatory conditions.

2. It has been found under a great variety of dynamic states of the circulation, and irrespective of the location of the cannula *in the ventricular cavity*, that the contour and pressure value of the systolic portion of the arterial curve reproduces accurately the ventricular pressure pulse.

3. Ventricular pressures considerably in excess of the aortic or carotid can indeed be obtained but only by placing the ventricular needle so that its opening is partially or wholly occluded during systole. Such curves are believed to represent technical artefacts and not the true pressure variations in the ventricle.

4. Basal arterial blood pressures from normal well trained dogs are not as high as generally supposed but give values approximating 124/85 mm. Hg.

REFERENCES

(1) Goldblatt, H., J. Lynch, R. F. Hanzal and W. W. Summerville. J. Exper. Med. **59**: 347, 1934.

(2) Hamilton, W. F., J. Brewer and I. Brotman. This Journal **107**: 427, 1934.

(3) Hamilton, W. F. and F. S. Brackett. This Journal **112**: 130, 1935.

(4) Hamilton, W. F., R. A. Woodbury and H. T. Harper. J. A. M. A. **107**: 853, 1936.

(5) Wiggers, C. J. Pressure pulses in the cardiovascular system. Longmans, Green and Co., New York, 1928.

CORD POTENTIALS IN THE SPINAL CAT[1]

JOSEPH HUGHES, G. P. McCOUCH AND W. B. STEWART

From the Pennsylvania Hospital for Mental and Nervous Diseases and the Department of Physiology, University of Pennsylvania, Philadelphia, Pa.

Received for publication August 25, 1936

Electrical changes in the cat's spinal cord have been studied by Gasser and Graham (8) and by Hughes and Gasser (10 and 11). They confined their attention to the potential picture which results from the setting up in dorsal roots of single and double volleys and to the effect upon that picture of backfiring an antidromic volley into the ventral horn cells. With recording leads placed longitudinally on the dorsal surface of the cord, the response to a single reflex volley consists of a negative spike followed by a longer and lower negative complex which is usually succeeded by a prolonged positive wave. With two synchronous volleys in adjacent dorsal roots, the spikes sum arithmetically; the succeeding components, on the contrary, fall far short of the arithmetic sum. Yet these convergent potentials are unaffected by an antidromic volley. On these and other grounds, the spike has been attributed to afferent fibers, the succeeding components to internuncial neurons.

Whether the internuncial potentials arise solely from fibers or also from cell bodies is not known. Adrian (1) and Eccles (5) have presented evidence suggesting the existence of cell potentials. We believe the results to be presented here afford a further basis for conjecture. Let us consider briefly what might be expected in the case of either alternative.

Were the source of cord potentials confined to axones, which respond in an all-or-none manner, the reduction of a second volley by a preceding one should affect negative and positive components alike. Furthermore, in view of the extent of convergence indicated by the occlusion of internuncial potentials resulting from synchronous submaximal volleys in adjacent roots, minimal volleys, which produce reflex facilitation, should give a corresponding degree of facilitation of internuncial fiber potentials. This relation should obtain in both spatial and temporal facilitation.

On the other hand, if central excitatory state be associated with a negative cell potential and central inhibitory state with positivity of cells, each susceptible to gradation of subliminal intensity, a thesis for which Eccles

[1] Aided by a grant from the Rockefeller Foundation to the Pennsylvania Hospital for Mental and Nervous Diseases.

has advanced strong evidence (5), the electric picture should deviate from such correspondence with the reflex as recorded myographically. The moiety of potential associated with central excitatory state of cells should not exceed the arithmetic sum of that due to the two volleys in isolation, and may well fall short of that value as a consequence of immediate reduction of central excitatory state as a result of discharge. This reduction should be greater in temporal than in synchronous spatial facilitation, since the early elements of the second volley impinge upon cells already charged with considerable C.E.S. and hence ripe for discharge.

This argument depends upon two assumptions: 1, that subliminal C.E.S. persists for several milliseconds in the absence of discharge; 2, that it is markedly and immediately reduced as a result of discharge. Strong evidence in support of both assumptions has been adduced by Eccles (3) and by Eccles and Sherrington (6).

METHODS. The method used for recording cord potentials was that employed by Gasser and Graham (8). The lumbo-sacral region of the cord was widely exposed and recording leads were placed on its dorsal surface, the ground lead about 1 cm. below the grid lead and a similar distance above the entrance zone of the roots stimulated. The leads consisted of camel's hair brushes moistened with Ringer's solution and impaled upon chlorided silver wire. A 4-stage amplifier, coupled by condensers of 2 μ.f. and with 2 megohm grid leaks fed a cathode ray oscillograph. Coreless induction coils stimulated dorsal roots by single break shocks. Isometric reflex responses were recorded photographically by a frictionless double torsion myograph from tibialis anticus and gastrocnemius muscles of the left leg. Save for these two muscles both lower extremities were denervated except for muscles inserting near the upper end of the femur, which were severed near their insertions. Each response was synchronously recorded by oscillograph and myograph.

Cats were the only animals used. The spinal cord was transected under general anesthesia several hours or weeks in advance. Preparation for recording included decerebration under ether.

RESULTS. In nine acute and five chronic cats the reduction in the ipsilateral flexor reflex by a preceding contralateral volley was correlated with the reduction in the first wave of the negative complex of the internuncial potential. Figure 1, in which both variables are plotted against the stimulus interval, is characteristic of the entire series. The approximate proportion between this wave and the response of tibialis anticus is consistently present in all cases of inhibition. It does not apply, however, to temporal facilitation, as is indicated by the earliest point on the graph.

Figure 2 illustrates another relation obtaining in all these animals: that between the degree of inhibition and the depth of positivity obtaining at the onset of the potential of the ipsilateral volley. Of the entire

series, figure 2 presents the case in which this proportion is most regular. Although it is true that the depth and duration of inhibition can be predicted approximately from the form of the positive wave, the variation from strict proportionality is usually far greater than in this graph. In chronic animals the ratio of inhibition divided by positivity is greater than in acute preparations.

Inhibition differentiates sharply between the negative and positive complexes. The first wave of the internuncial potential is more resistant to it than any of the succeeding components. Even when this crest is only moderately reduced, the positive wave is usually wiped out com-

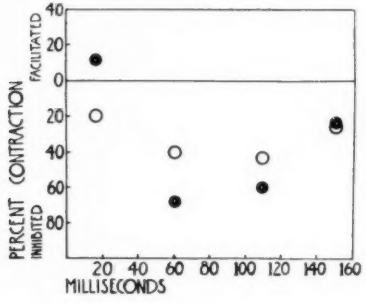


Fig. 1

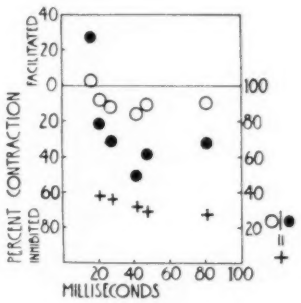


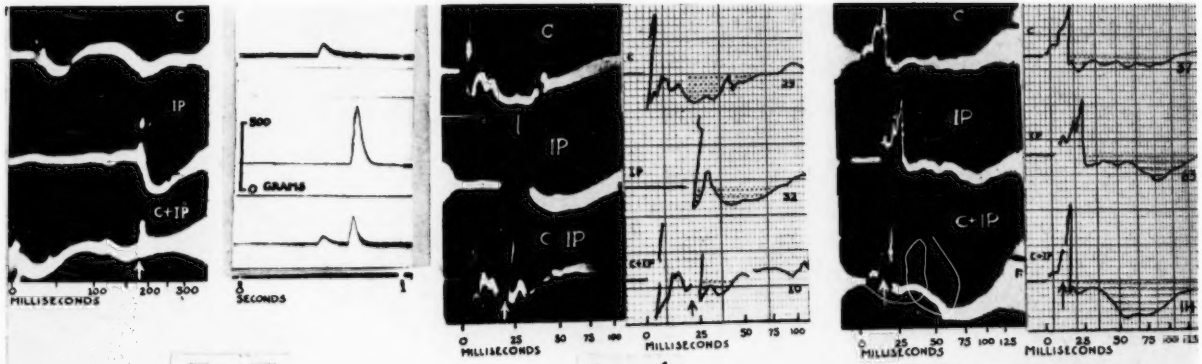
Fig. 2

Fig. 1. Acute cat. Ordinate below the base line of zero potential indicates percentage reduction in first negative crest of the internuncial potential (open circles) and percentage contraction inhibited (black circles). Percentage contraction facilitated is indicated above the base line. Abscissa indicates interval between contralateral and ipsilateral stimuli in milliseconds.

Fig. 2. Acute cat. Ordinate, abscissa, and black circles as in figure 1. Open circles indicate in arbitrary units the potential following the contralateral volley obtaining at the time of the ipsilateral shock. Negativity is indicated above, positivity below the line of zero potential. Crosses indicate the ratio of positivity obtaining divided by percent contraction inhibited in percentage indicated at the right of the figure.

pletely. In three acute cats not only is the positivity attributable to the ipsilateral volley eliminated, but even the residual positivity from the contralateral volley is reduced or abolished (figs. 3 and 4). On the other hand, in three chronic preparations characterized by a reversion of the crossed reflex, following free exposure of the cord, from the extension typical of the undissected chronic spinal cat to a powerful crossed flexion, positivity was increased at short intervals and in one case at all intervals (fig. 5).

Summation was studied in five experiments. Spatial facilitation was elicited by minimal shocks to two strands of the same dorsal root tested


C
IP
C+IP
MILLISECONDS
SECONDS
FIG. 3
FIG. 4
FIG. 5
BOTH
TIME 1/60 SECONDS
TIME 1 SECOND
FIG. 6

Figs. 3 to 6

first in isolation and then in synchronous combination. The combined response developed a negative complex exceeding in crest height and often in area the sum of the negativity of the two single responses (fig. 6). Yet the extent of this excess was uniformly less than the degree of facilitation measured by the myograph.

In sharp contrast is the result of temporal facilitation. Whether tested by two successive volleys in the same afferent fibers or by following a contralateral by an early ipsilateral volley, the negativity attributable to the second volley was invariably less than when the second shock was given alone (figs. 7, 8, and the earliest point in fig. 1).

Discussion. The experiments upon crossed inhibition of the ipsilateral flexor reflex accord with those of Hughes and Gasser upon ipsilateral inhibition in two important respects: the reduction of the crest height of the first wave of the negative complex in proportion to the reduction of the reflex response and the dependence of this reduction upon the degree of positivity obtaining as a result of the conditioning shock. The reduction of the negative complex indicates that the site of inhibition in these cases is chiefly at the internuncials. This observation affords an obvious explanation for the immunity of such inhibition from the effect of an antidromic volley reported by Eccles and Sherrington (7). It confirms the earlier conclusions of Lorente de Nó (12) (13) (14) (15).

The relation between positivity and inhibition is in accord with Eccles' observations upon superior cervical ganglion and supports the interpretation he has offered (5). On the other hand, Gasser (9) has suggested an explanation based upon the late subnormal period associated with the positive after potential of fibers.

Fig. 3. Acute cat. Cord potentials (left) and reflex responses (right) following a single break shock to a contralateral dorsal root (upper row); to an ipsilateral dorsal root (middle row); to contralateral followed by ipsilateral (lower row). In this and subsequent figures reflex responses are from tibialis anticus. Time in myograph records is in seconds, in oscillograph records in milliseconds. The standardization is in grams and applies to all myograms. Stimulus interval 187 m.s. Note obliteration of positivity. Myograph records retouched.

Fig. 4. Acute cat. Potentials as in figure 3. In the right hand column these are traced on semilogarithmic paper and the area of positivity occurring after the ipsilateral shock is computed in arbitrary units indicated by numerals and stippling. Stimulus interval 22 m.s. Note reduction of positivity.

Fig. 5. Chronic cat. Potentials and tracings as in figure 4. Stimulus interval 8 m.s. Note summation of positivity.

Fig. 6. Acute cat. Two instances of synchronous spatial facilitation from adjacent strands of the same dorsal root. In the tracings in the center column the area of the negative complex is computed in arbitrary units indicated by stippling and numerals. In this and subsequent potential records, the time sweep is linear and time is indicated in 1/60 second. In the combined response of the left hand series, note the spike potential surmounting the crest of the slower (cell?) potential and the abrupt decline thereafter.

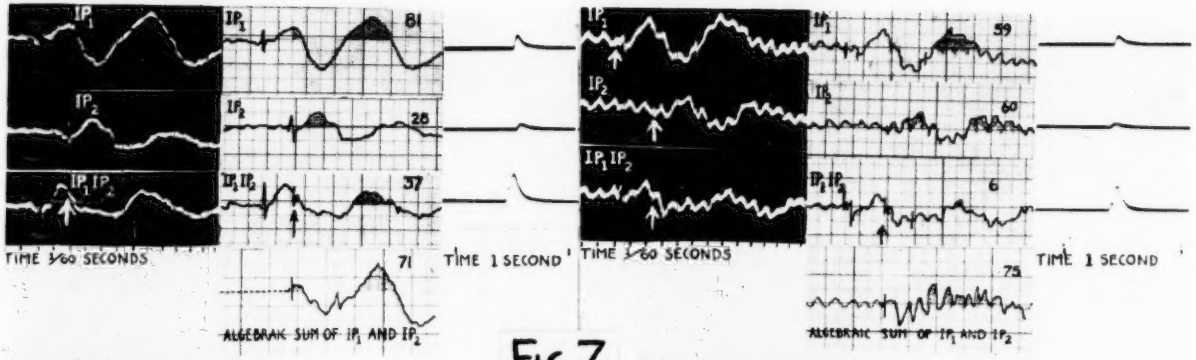


FIG. 7

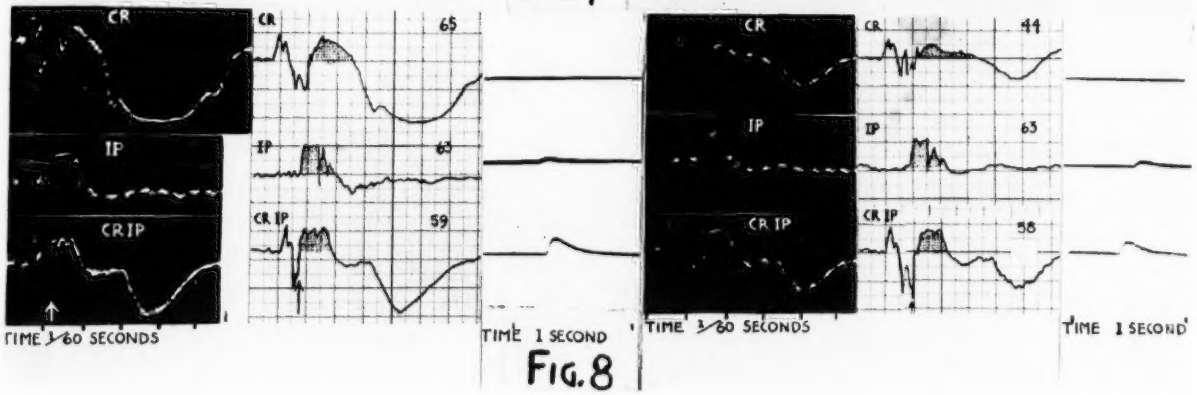


FIG. 8

Figs. 7 and 8

In one respect our observations upon crossed inhibition differ from those of Hughes and Gasser (11) on ipsilateral inhibition. This concerns the fate of the positive wave following the test shock. Even at intervals at which the negative complex is little reduced, the positive wave is almost completely obliterated. Not only is the positivity associated with the ipsilateral volley wiped out, but sometimes also the residual positivity from the contralateral volley. This result may be correlated with the observation of Eccles and Sherrington that an ipsilateral reflex may be protected from contralateral inhibition when preceded by an intercalated ipsilateral volley (7). It may be discussed more profitably after considering the site of production of the positive wave.

The combination of earlier work with the present results affords a basis for tentative correlation of the two major components of the internuncial potentials with reflex events in the cord. The first wave of the negative complex is reduced proportionally to the reduction in the ipsilateral flexor reflex whether that reduction be from contralateral (fig. 1) or ipsilateral inhibition (Hughes and Gasser, 11). It is increased when ipsilateral flexion is facilitated by synchronous stimulation of two strands of a dorsal root (fig. 6). It may be ascribed with assurance to the internuncial units activating flexion located in the ipsilateral dorsal horn and at its base.

The relation of the positive wave to the flexor reflex is as definite as that of the negative complex, though opposite in sign. The depth of reflex inhibition bears a rough proportion to the depth of positivity obtaining at the time of the test volley, whether the conditioning stimulus be ipsilateral (Hughes and Gasser, 11) or contralateral (fig. 2). Yet the relation is far from regular, presumably owing to the overlap of positive and negative components. Since the flexor reflex is inhibited in the presence of positivity following either an ipsilateral or a contralateral stimulus, we attribute most of the positive wave to a central inhibitory state in the internuncial perikarya in the region of both dorsal horns. We are inclined to allocate the major share of its later components to the contralateral horn, since this site affords the only explanation that has occurred to us for the virtual obliteration of positivity from the ipsilateral volley by a contralateral volley preceding it by a considerable interval, and for the

Fig. 7. Acute cat. Two instances of temporal facilitation from two volleys in the same dorsal root fibers. In this and the next figure negativity is plotted from the time of the second volley. The lowest plot in each central column is a reconstruction of the algebraic sum of the two volleys, assuming no convergence. Stimulation intervals: 25 m.s. (left) and 33 m.s. (right). Note the reduction in the potential from the second volley.

Fig. 8. Acute cat. Two instances of temporal facilitation from a contralateral followed by an ipsilateral volley. Stimulus intervals: 9 m.s. (left) and 12 m.s. (right). Note the reduction in the potential from the second volley.

exceptional case of the response at short intervals after a large crossed flexion reflex which induces an *increase* in positivity following the subsequent ipsilateral volley.

Let us consider first the commoner case in which extension is the dominant crossed effect. The inhibition curve (fig. 1) and other evidence (Mathes and Ruch, 18; McCouch, Snape and Stewart, 19) indicate that this situation obtains as a concealed reflex at long intervals even in cases where a crossed flexion of briefer latency is the only obvious contralateral response. Anatomically the crossed and uncrossed axones of internuncial cells are known to branch, sending collaterals to both dorsal and ventral horns (Ramon y Cajal, 20; Lorente de Nó, 17). Let us assume that the crossed collaterals ending dorsally are in most instances inhibitory, those ending ventrally excitatory. The positive wave following the contralateral volley is presumed to indicate central inhibitory state chiefly in the flexor units of the ipsilateral dorsal horn. The dominant effect of the ipsilateral volley is excitation of these units. The conflict results in diminution of ipsilateral negativity and of the residual positivity from the contralateral volley. Since the cells of the ipsilateral horn are inhibited, they give rise to no activity in fibers crossing to end in the contralateral dorsal horn. Hence the ipsilateral volley fails to develop a positive wave.

Such is the usual picture. In three chronic preparations, however, in which contralateral stimulation induced an exceptionally large crossed flexion reflex, at short intervals positivity was enhanced. Here we must regard flexion as the dominant crossed reflex. At the ipsilateral dorsal horn, it will inhibit extensor units. These are also inhibited by the ipsilateral volley, and positivity from one source adds with that from the other. Since the crossing internuncials stimulated by the ipsilateral volley are facilitated by the preceding contralateral, a further increment of positivity develops at the contralateral dorsal horn.

Our experiments upon facilitation indicate a salient difference between the case where two strands from a dorsal root are stimulated synchronously and where two volleys are set up successively in one strand. In the first instance, the negative complex exceeds the sum of that of each of the two components. In the second instance it is far less than the sum.

We are not the first to demonstrate the absence of summation in the negative complex of the cord potential in cases of reflex temporal summation. Hughes and Gasser (11) obtained similar results which they attributed to convergence of long circuits of the first volley with short circuits of the second at a point downstream from the internuncials recorded. It is most likely that such concealed convergence occurs, but it is not necessary to assume it. If we admit the existence of subliminal cell potentials of appreciable duration that are depleted abruptly upon discharge of a nerve

impulse, the observed difference between spatial and temporal facilitation accords with expectation. In synchronous stimulation of two strands of a root, the volleys converge upon cells most of which are at rest and require the development of their total threshold potential for discharge. Hence the cell potential recorded may approximate the arithmetic sum of that from each of the two volleys separately. In temporal facilitation by two volleys in the same strand, on the other hand, it is only the first volley which meets such inactive cells which it charges almost to threshold. The second volley adds but a small further increment of potential before the perikarya discharge and are thereby depleted of potential. The distance between the point of stimulation and the cells of the dorsal horn is too short to admit of appreciable temporal dispersion. Consequently, the residue of the second volley falls during the absolutely refractory phase and fails to develop a cell potential. This accounts for the failure of the second volley to develop a potential equal to that which it yields when not preceded by a previous volley (fig. 7).

Were perikarya the only source of potential there is no obvious reason why the negativity of the combined response should ever exceed the sum of that of the two components, as it does in spatial facilitation. The excess must be attributed to fiber potentials. It is noteworthy, however, that even in this case, the ratio by which the combination exceeds the sum of the components is consistently less in the case of the negative complex than in that of the reflex response.

Within the past year a brilliant paper by Lorente de Nó (16) has presented the problem of synaptic excitatory process from a point of view at variance with that urged here. With stimulation of fibers in the floor of the fourth ventricle and recording from the internal rectus muscle he has obtained temporal facilitation at intervals from 1.5 to 80 m.s. but at briefer intervals a combined response less than that to the testing shock alone. At short intervals the test shock finds refractory those fibers which have just responded. The facilitation is attributed to delay paths including side circuits affording a continuous round of re-excitation. The initial depression is ascribed to the subsidence of synaptic excitatory process from the conditioning volley before the development of that from the testing volley.

On the other hand, with motoneurons of tibialis anticus Eccles found that an antidromic volley reaching the cord 15.3 m.s. before the fastest impulses in the second of two reflex volleys reduced the facilitation of the last volley by the first, although it failed to do so when it reached the cord only 1.8 m.s. before the first volley (3). The inference appears convincing that in the absence of the antidromic volley, early increments of C.E.S from the first volley must have persisted for several m.s. in the cells of the ventral horn. Moreover, in the case of sympathetic ganglia, where

there is no evidence of delay pathways, Bronk, Tower, and Solandt (2) and also Eccles (4) have recorded facilitation enduring for much longer periods.

The data in the present paper appear to favor the view that increments of central excitatory and of central inhibitory states persist for many milliseconds upon the surface of perikarya and are associated respectively with negative and positive potentials which constitute the major portion of the electrical changes recorded from the spinal cord.

In conclusion, we cannot stress too strongly that our fragmentary interpretation of cord potentials is offered not as a unique solution of a complex problem, but merely as a tenative hypothesis to be tested by further evidence.

SUMMARY AND CONCLUSIONS

Potentials have been recorded from the dorsal surface of the cord synchronously with reflex responses myographically recorded in acute and chronic spinal cats.

Crossed inhibition of the ipsilateral flexor reflex is associated with an approximately proportional reduction of the first negative crest of the internuncial complex.

This inhibition is roughly proportional to the depth of positivity obtaining at the time of the ipsilateral volley.

Hence the principal site of this inhibition is internuncial.

Even at stimulation intervals at which the negative complex is little reduced, the positive wave due to the ipsilateral volley is usually obliterated and even the residual positivity from the contralateral volley is frequently reduced.

Synchronous spatial reflex facilitation is associated with facilitation of the negative internuncial complex.

Temporal reflex facilitation is associated with reduction of the negative internuncial potential from the second volley.

These results are tenatively interpreted upon the assumption that negative and positive cell potentials contribute to the electrical changes recorded.

REFERENCES

(1) ADRIAN, E. D. J. Physiol. **72:** 132, 1931.
(2) BRONK, D. W., S. S. TOWER AND D. Y. SOLANDT. Proc. Soc. Exper. Biol. and Med. **32:** 1659, 1935.
(3) ECCLES, J. C. Proc. Roy. Soc. **107 B:** 557, 1931.
(4) ECCLES, J. C. J. Physiol. **85:** 207, 1935.
(5) ECCLES, J. C. J. Physiol. **85:** 464, 1935.
(6) ECCLES, J. C. AND SIR CHARLES SHERRINGTON. Proc. Roy. Soc. **107 B:** 586, 1931.
(7) ECCLES, J. C. AND SIR CHARLES SHERRINGTON. Proc. Roy. Soc. **109 B:** 91, 1931.

(8) GASSER, H. S. AND H. T. GRAHAM. This Journal **103**: 303, 1933.
(9) GASSER, H. S. Johnson Foundation Lectures, Univ. of Penna. Press, 1936.
(10) HUGHES, J. AND H. S. GASSER. This Journal **108**: 295, 1934.
(11) HUGHES, J. AND H. S. GASSER. This Journal **108**: 307, 1934.
(12) LORENTE DE NÓ, R. Monatsschr. f. Ohrenheilkunde **61**: 1066, 1927.
(13) LORENTE DE NÓ, R. Ergebn. Physiol. **32**: 216, 1931.
(14) LORENTE DE NÓ, R. Trans. Am. Otol. Soc. **22**: 287, 1932.
(15) LORENTE DE NÓ, R. This Journal **103**: 704, 1933.
(16) LORENTE DE NÓ, R. This Journal **113**: 505, 1935.
(17) LORENTE DE NÓ, R. Personal communication.
(18) MATTHES, K. AND T. C. RUCH. Quart. J. Exper. Physiol. **22**: 221, 1932.
(19) MCCOUCH, G. P., W. J. SNAPE AND W. B. STEWART. This Journal **111**: 263, 1935.
(20) RAMON Y CAJAL, S. Histologie du systeme nerveux, Paris, 1909, Volume I, p. 344, fig. 126, and p. 390, fig. 141.